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Investigations on the Effect of Phosphoric Acid Esters on Pests occurring in Stone Fruit (*Rhagoletis cerasi* L., *Laspeyresia funebrana* Tr., *Hoplocampa minuta* Christ. and *Hoplocampa flava* L.)*

By Dr. Gerhard Sch u h m a n n

Paper from the Institute for Fruit and Vegetable Growing at the Federal Biological Station Heidelberg (Director: Oberregierungsrat Dr. H. Thiem)
and the Institute for Plant Protection at the Agricultural College, Hohenheim (Director: Prof. Dr. B. Rademacher).

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* Extract from the thesis of Sch u h m a n n (21).

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A. Formulation of the Question

The phosphoric acid esters hold a position of high rank among the modern insecticides used in fruit growing. They penetrate into the plant tissue, and can be applied successfully against a number of harmful insects which often cannot be controlled with other insecticides. Of the phosphoric acid esters, diethyl-p-nitrophenyl thiophosphate ("E 605") is today used extensively in the whole field of fruit growing, as well as to control many other pests occurring in agriculture. According to Schrader (22), the annual production all over the world in 1951 amounted to 3000 tons (calculated on active ingredient), and a further increase is expected. Special consideration has been given to this compound in this paper. It has frequently been singled out as a prototype from among the group of phosphoric acid esters.

Despite the extensive scale on which phosphoric acid esters are used and the wide range of experience gained from their application, there are still a number of important questions to be answered. The insecticides containing phosphoric acid esters could not be employed everywhere with the desired amount of certainty; e.g. Thiem (32, 34) was most successful in combatting the cherry fruit fly (*Rhagoletis cerasi*) occurring on cherry trees and on fly honeysuckle but he also had to put up with a number of serious failures for which he could not easily account. For instance the effect of phosphoric acid esters on larvae of the plum sawfly already present in the fruit was not clarified satisfactorily. The same applied to the range of action of the esters on the various stages of development of the plum fruit maggot, and also to the question of a suitable control date.

In this paper the effect of phosphoric acid esters on these pests has been closely investigated in order to establish the cause of failures met with during

the control of the above pests so that these can be remedied in field work. Attention was concentrated chiefly on the behaviour of the phosphoric acid esters on the fruits. With the aid of results already gained, small-scale tests (and wherever possible large-scale tests) were carried out to prove the correctness of information obtained.

In the investigations, the following phosphoric acid esters were given primary consideration:

1. "E 605" f with 50 % diethyl-p-nitrophenyl ester of thiophosphoric acid;
2. "Systox" (E 1059, 8169), containing 50 % diethyl thiosphosphoric acid ester of ethyl thioglycol ether;
3. "OMPA", with 66 % octamethyl pyrophosphoric acid amide as active ingredient.

B. Longevity of phosphoric acid esters

Before the preparations were tested outdoors, they were first submitted to a laboratory examination. These preparations were on the market under the designations "E 605" f and "E 605" conc. (1947—1952). They contained diethyl-p-nitrophenyl thiophosphate as the major part of active ingredient. There were no differences in the LD₅₀ value established in the film test process with *Calandra granaria* L. (Schuhmann 21). This would mean that the product does not lose any of its efficacy even when stored for a long period.

C. Methods of tracing the phosphoric acid esters with *Drosophila melanogaster* M.

I. Breeding of *Drosophila* and general data on test methods

The phosphoric acid esters were traced with the aid of a strain of *Drosophila melanogaster* M.

The *Drosophila* were bred in 1 litre flasks at 24°C. Preparation of the nutrient medium: 125 g syrup and 710 g water were boiled with 10 g agar, and replenished with 5 ccs of a 20 % alcoholic solution of "Nipagin" M and 130 g of corn flour swollen in 200 g of water. The nutrient medium was poured into the breeding flasks while it was still warm and liquid. After it had become cold, several drops of a suspension made of baker's yeast were poured over it. Each flask contained about 400 puberal females and the same number of males for a period of 12 hours for the purpose of obtaining eggs.

Since the method of bioassay of phosphoric acid esters was found to be dependent upon temperature and humidity, the tests were carried out uniformly in a thermoconstant room at 20°C and with a relative humidity of 90—95 %.

In those tests in which parts of fruits were tested for their ester content, the *Drosophila* flies were kept without nutrition for 12 hours before starting the tests so that when they were placed on the poisoned fodder they would immediately begin to eat thus coming into intimate contact with the insecticidal residues. The insertion of the flies was carried out under an ether narcosis. The ether used for this purpose must be pure. In order to prevent the formation of insecticidally effective peroxides, a reducing solution of 1 g ferrous sulphate in 10 ccs water was added to 100 ccs diethyl ether and shaken several times.

After being thoroughly rinsed in soda water and alcoholic lye, the Petri dishes and glass plates used in the tests had to be heated to 180° C for 2 hours to decompose any adhering residues of the agent.

The acetone used for extraction could not be completely separated from diethyl-p-nitrophenyl ester even following two redistillations. It was unfit for use in further tests.

In all the tests the flies were submitted to uniform electric illumination. Since the phototropic reaction of *Drosophila melanogaster* is positive, the test dishes were covered with filter paper to disperse the incident light. The flies then distributed themselves evenly.

II. Determination of the time taken by 50 % of the *Drosophila* flies to assume an irreversible back position

In experiments where a large number of test animals are used it is difficult to read off directly the time for 50 % mortality. (For the sake of simplicity it will be called the T 50 % value.) If it is required to read off this value directly it would be necessary to check the tests constantly, which however would take up too much time particularly in tests lasting several days. This is saved by calculating the T 50 % values from the mortality curve according to the Probit method of Bliss (1). In this way the number of flies which died or which were on the verge of death was ascertained in time intervals resulting from the toxicity of the substance being tested.

For our purposes we were quite satisfied with the graphic representation which could be plotted relatively quickly. The mean error of the T 50 % values determined by the Probit method was less than when the 50 % mortality was read off directly. This is understandable when it is considered that for one evaluation at least 10 individual checks counted at different times were carried out. If the points are in a straight line they not only show whether the given function of time is normally distributed but also give direct, when it is normal, a graphic mean value of the reaction period, the standard deviation and the time in which any percentage of the flies between 0 and 100 % had reacted. In the tests in which, due to the slight proportion of insecticide, 100 % mortality was not obtained, the straight line should show a break in the upper percentage range. In these cases the upper part was left unconsidered.

The following tests were carried out to determine the susceptibility of *Drosophila melanogaster* of different ages to diethyl-p-nitrophenyl thiosphosphate:

III. Age of *Drosophila* flies used in the tests

After pouring in a layer of the described nutrient medium 3 cm deep, 400 flies of which about 50 % were females were kept 6 hours in each cylindrical breeding flask (capacity of 250 ccs). Breeding was carried out in a thermostat at 24° C. Hatching of the flies began 11 days after the eggs were laid, and lasted 48 hours. The first imagoes were removed 24 hours after they appeared and placed on fresh nutrient medium so that there was a difference of no more than 24 hours between the ages of the flies in each flask.

The same stock solution of "E 605" was used in each test. 50 mg "E 605" conc. and 500 mg Turkey red oil were put in a graduated flask and filled up to

250 ccs with acetone. To prepare the tests which lasted from 1 to 14 days after the flies had hatched, 1 cc of stock solution was pipetted each time onto a round filter (Schleicher-Schüll SS 595) with a diameter of 9 cm. The filters were left to dry on a glass base for 15 minutes in a laboratory room at 24° C, after which they were rolled together and placed in a test tube. 25 non-anesthetized flies were placed in each test tube by means of a glass tube. The glass tubes, sealed with a cellulose stopper, were kept in a rack at an angle of 45° so that the damaged flies fell to the bottom of the test tubes and could be easily counted. After the first flies had been damaged, the process was noted every 2 minutes until 100 % of the flies had reacted, in other words until there was not a fly left capable of exercising regular movements.

The average values of 50 % mortality gained from five replications are shown in the graph in Fig. 1.

T 50% values expressed in minutes

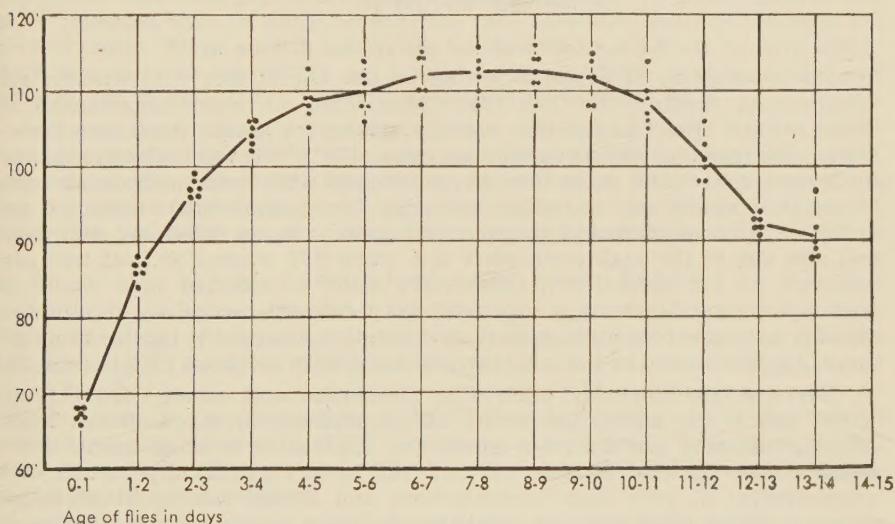


Fig. 1. 50% mortality of *Drosophila melanogaster* M. of various ages following effect of "E 605" conc. The average values are joined by the curve. One point represents 25 flies.

According to the graph the greatest amount of resistance was shown by the 6 to 10 day old *Drosophila* flies. Young and old flies are less resistant. In this test the very slight difference between flies aging from 6 to 10 days cannot be definitely checked statistically. The flies used in all the following tests were 7 to 10 days old. The difference in the age of the flies used in the individual tests and in those carried out for comparative purposes was 24 hours at the most.

D. The effect of phosphoric acid esters on *Rhagoletis cerasi* L.

I. Importance of *Rhagoletis cerasi*

In Germany, just as all over Central Europe, the cherry fruit fly is one of the most important pests in cherry growing. It is particularly among the late cherry varieties that the danger of maggot infestation is very great. An infestation of 80 to 100 % is no rarity. Maggoty cherries cannot be used as dessert cherries or for canning purposes. Cherry varieties used for distilling which normally remain on the trees longer than other varieties fall prematurely when infested by maggots due to an inclination to rot. The profitableness of growing cherries becomes a doubtful matter when the value of such fruits is decreased (Thiem 32).

II. Position with regard to control

Control possibilities are offered against

1. the pupae in the soil
2. the imagoes
3. the eggs and larvae.

1. The control of pupae in the soil

The eradication of pupae occurring on the soil surface with suitable soil disinfectants, chiefly with fruit tree carbolineum oil, was recommended by Wiesmann (37). Thiem (26) tested a number of agents (fruit tree carbolineum oil, pyridine, tetrachloroethane, inter alia) which were effective against the pupae, but at the same time he pointed out that such methods are only of practical significance in exceptional cases. Their application miscarried due to the versatile utilization of the soil with under-cultures which are destroyed, and also due to the high costs, since e.g. when 8 % washes of fruit tree carbolineum oil are applied, ten times more water is required than would be used in spraying. Moreover a successful soil treatment carried out locally does not offer adequate protection against an infestation because the passive propagation of the flies caused by the wind can assume large proportions (Thiem 26, 32).

Thiem (35) also used, inter alia, phosphoric acid esters ("E 605" f and "E 605" conc.), and agents containing dichlorodiphenyl trichloro-ethane, hexachlorocyclohexane and dinitro-o-cresol. The application of these agents a few weeks before the flies hatched did not lead to any satisfactory results even when applied in very high concentrations and at the rate of 34 litres per sq. metre. Their effect was also unsatisfactory when the pupae were immersed in the different preparation washes. Schneider and Vogel (19, 36) also tested agents containing diethyl-p-nitrophenyl thiophosphate, hexachlorocyclohexane, dichloropropane and dichloropropene for their usefulness in killing the pupae, and they confirmed that the effect was unsatisfactory.

Various methods of soil processing designed to prevent the flies from hatching were also insufficiently effective (Thiem 28).

2. The control of imagoes

The old methods (Sprengel 25, Thiem 28, Wiesmann 37) of using sodium flouride, nicotine, derris pyrethrum and other agents are no longer of any significance now that people are acquainted with the modern contact insecticides. Thiem (28) made a vain attempt with 89 substances to actively attract

the flies in order to kill them with added poisons. His tests to trap the flies by means of light or to kill them on the ground immediately after they had hatched did not produce any useful results because in the latter case the spray deposits were washed off relatively quickly by dew and rain. Even when the more modern contact insecticides such as dichlorodiphenyl trichloroethane, hexachlorocyclohexane, chlordane and diethyl-p-nitrophenyl thiophosphate are used, this method is far from promising for the same reason (Thiem 35, Vogel 36).

The most successful method employed today is the control of imagoes on the tree. For this purpose, agents containing dichlorodiphenyl trichloroethane ("DDT") are preferred because their effect lasts much longer than that of agents containing hexachlorocyclohexane and phosphoric acid esters. This method, however, still leaves a lot to be desired. Firstly compact areas have to be treated because a passive flight over large stretches is possible and the flies are able to lay eggs on treated trees before they succumb to the poison. The result of this is that unsurmountable difficulties are often encountered in cherry growing districts divided into a large number of plots and where the location is inaccessible to spraying equipment. Moreover ordinary sprays have to be repeated once or twice because the flies normally continue hatching for several weeks. When several sprays are applied the cherries are heavily soiled. With one spray 22 mg "DDT" are applied to 1 kg of fruit (Schneider and Vogel 19). In the USA 7 mg/kg is permitted for saleable fruit.

New methods tested by Thiem (34) have considerably simplified the treatment by using rain-proof "DDT" as aerosol. A single treatment was sufficient to prevent the flies from laying eggs for several weeks. A disadvantage of this is the necessity of purchasing new equipment although this is almost balanced out by the possibility of also using such equipment as atomizers.

3. The control of eggs and larvae

The advantages of a possible control of eggs and larvae of the cherry fruit fly are obvious. It would be possible to treat individual trees, and cherries free of maggots could be harvested irrespective of the state of neighbouring trees. In addition the prognosis for a treatment could be given more easily in this case than in the application of "DDT" since egg laying can be determined relatively easily whereas a "DDT" treatment must already have been carried out by this time. Moreover the control could not be delayed so easily because of bad weather and holidays.

Thiem (35) has been carrying out tests with "E 605" on *Lonicera* bushes and sweet cherries regularly since 1946. These show that the ester can prevent the cherries becoming infested by maggots provided the flies have already laid eggs. At the same Institute, Lüdicke (13) investigated the influence of "E 605" f on fly honeysuckle berries colonised by cherry fruit fly eggs, and he found that a strikingly low number of pupae developed in the fruit. The proportion of pupae from treated berries to those from untreated berries was as follows: for an application of 0.03 % "E 605" f 14 : 103, for 0.05 % "E 605" f 1 : 100 and 2 : 44. In this investigation no check was made as to whether it was the eggs or the hatched larvae that were first killed by the agent. Thiem (31) was able to obtain satisfactory results in large-scale tests carried out on sweet cherries with "E 605" f in 1949 (Giedensbach) and with "E 605" conc. in 1951 (Seeheim). Schöne (20) wrote of partial success gained with "E 605" f in 1951. From the USA there is a report by Cox (12) of a 100 % success with an application of "Parathion" (active ingre-

dient: diethyl-p-nitrophenyl thiophosphate) against *Rhagoletis fausta* O.S. and *Rhagoletis cingulata* Lw. which are closely related to *Rhagoletis cerasi* L. In 1947 and 1948, following double treatments carried out at intervals of 7 and 13 days, not a single living larva was found.

With the position as such, the success of an "E 605" treatment appeared guaranteed. It required, however, an explanation of the effect in detail. The object of carrying out thorough investigations, therefore, was to answer the questions:

1. How does diethyl-p-nitrophenyl thiophosphate react on the fruits?
2. Does the compound have an effect in the fruit on eggs and larvae?

III. The behaviour of diethyl-p-nitrophenyl thiophosphate on and in the cherry following application

1. Residual effect of phosphoric acid esters on the fruit peel of the cherry

In each control two cherry trees of the same variety were treated with 0.03 % "E 605" conc. Approximately 15 litres per tree were applied uniformly by means of a high pressure sprayer. The trees were 9 years old.

Treated varieties and dates of treatment:

Variety	Degree of ripeness	Date and time of treatment	
1. Kunzes Kirsche	yellowish-green, hard	13. 6. 51	5 p.m.
2. Spanische Weiße	green (14 days before the harvest)	22. 6. 51	11 a.m.
3. Schöne aus Chatenay (late sour cherry)	ripe	27. 6. 51	5 p.m.

The toxic residues were tested at different intervals. From thinly peeled cherries (about $\frac{1}{2}$ mm), peel parts from about 8 half cherries (about 2 g) were placed under a Petri dish with 100 flies on top. The individual tests were repeated four times. Since no additional fodder was given to them, the flies were forced to consume the cherry juice adhering to the inside of the peel. The T 50 % values determined according to the Probit method are given in Table 2.

A guide to the absolute quantities of poison present was given by a series of tests carried out with fixed "E 605" concentrations. For this purpose untreated cherries were finely ground. "E 605" emulsions of certain dilution degrees were added to the cherry pulp in order to obtain the required concentrations. Beforehand the cherry pulp was heated for 30 minutes to 75° C to prevent a subsequent fermentative inactivation of the phosphoric acid ester. 2 ccs of the cherry pulp, which had been mixed with various quantities of ester and which had been shaken several times over a period of 2 hours, were placed in each Petri dish. The pulp was not spread out to avoid it drying up, with the result it could be used as nutrition for the flies during the whole of the test.

The average times obtained for 50 % mortality are listed in Table 2.

Table 1. Toxicity of diethyl-p-nitrophenyl residues in the cherry peels following "E 605" conc. sprays against *Drosophila melanogaster* M. The numbers indicate the average values of 50 % mortality in minutes with the true mean errors from four replications. In the next column the absolute active ingredient residue in mg/kg cherry peel is given, calculated from the calibration curve according to the values of 50 % mortality.

Start of test in days following treatment	Kunzes Kirsche treated 13.6.51		Spanische Weiße treated 22.6.51		Schöne aus Chateanay treated 27.6.51	
	50 % + in minutes	active ingredient mg/kg	50 % + in minutes	active ingredient mg/kg	50 % + in minutes	active ingredient mg/kg
1	104 ± 4	75.0	122 ± 6.6	51.0	112 ± 17.1	65.0
2	266 ± 16.8	13.5	655 ± 18.8	3.3	722 ± 31.2	2.8
3	528 ± 29.8	4.6				
4	—		1282 ± 73.7	1.2		
5	1046 ± 81.4	1.6			1506 ± 49.1	0.5
6	—		1680 ± 116	0.61		
7	—					
8	1556 ± 86.5	0.9	1842 ± 109	0.57	2325 ± 71.4	0.44
10					3207 ± 201	0.27
11			2562 ± 115	0.38		
12	2090 ± 113	0.5			3417 ± 110	0.25
15			4455 ± 275	0.16	5475 ± 245	0.12
17	4397 ± 340	0.15				

Table 2. Comparative series with given concentrations of "E 605" conc. in cherry pulp, and the average values of 50 % mortality for *Drosophila melanogaster* M. in minutes obtained in the biological test, with the true mean errors from 4 replications using 100 flies in each.

Cherry pulp		Average values of 50% mortality in minutes with the mean errors	Eradication of flies attained in %
"E 605" conc. concentration	Active ingredient mg/kg		
0.05000 %	250	59 ± 2.3	100
0.01000 %	50	127 ± 4.4	100
0.00500 %	25	170 ± 11.0	100
0.00100 %	5	525 ± 13.7	100
0.00050 %	2.5	781 ± 19.5	100
0.00010 %	0.5	2182 ± 218.0	40
0.00005 %	0.25	3395 ± 300.0	10

In the tests with low concentrations, there were no cases of 100 % killing (see last column), and 50 % mortality had to be determined here by extrapolation.

If the times of 50 % mortality are depicted as a function of the active ingredient content in the test pulp and shown as a coordinate system with logarithmic equivalents of the abscissa and the ordinate (Fig. 2), it is seen that the points of intersection in the lower part are on a straight line. At Point K (25 mg/kg, T 50 % 170 minutes) there is a break in the straight line which then rises steeply. I join him in his explanation that it is in the low concentrations that the active ingredient in the body of the insect is inactivated.

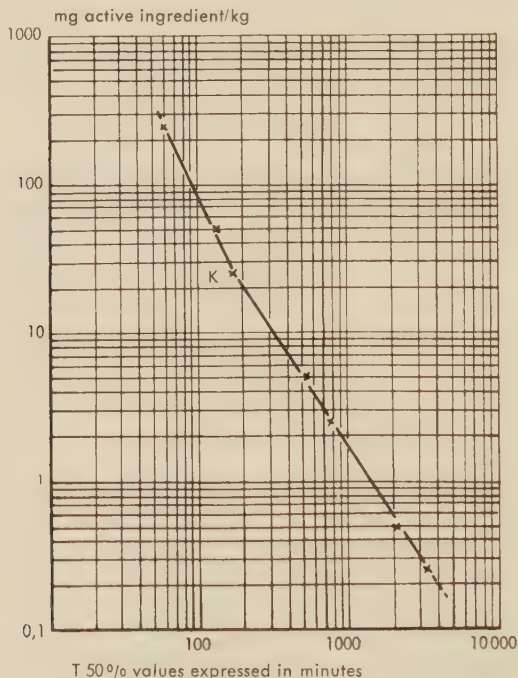


Fig. 2. Time of 50 % mortality in minutes of *Drosophila melanogaster* M. as a function of diethyl-p-nitrophenyl thiosphosphate content in mg/kg cherry pulp. Representation in logarithmic equivalents.

After calculating the straight lines in the lower part, it is possible to draw a conclusion with respect to the insecticidal content in the cherry peels from the function now known from the established mortality times (Table 1). From this, the active ingredient residues, also given in Table 1, were calculated in mg/kg.

In all probability the actual residues in the cherry peels are somewhat greater than is shown by the tests since the "E 605" dissolved in the lipides of intact peels is not taken orally by the flies, and the contact effect of the agent is reduced when it dissolves in lipides and similar compounds. The funda-

mental behaviour of "E 605", however, shows little change since at the most a parallel shift of the values occurs in the coordinate system.

From this it is realized that the toxicity of residues on cherry peel disappears more or less completely after 2 to 3 days, whereas any residue left over after this time loses its insecticidal efficacy at a considerably slower rate. No further residues of preparation whatsoever could be traced in the biological test 15 to 17 days following spraying. The three tests carried out at different times on three different varieties are more or less uniform. All in all, the deviations are so slight that they can hardly be of any significance in the practical effect against the cherry fruit fly. It was decided not to carry out a further investigation.

2. The penetrative property of phosphoric acid ester in the cherry fruit

Parallel to the tests, the penetrative property of the ester in the fruit was investigated. According to Frohberger (8) the compound does not penetrate into citrus fruits, apples, tomatoes, grapes and potatoes. Corresponding tests on cherries had not been carried out.

Method

Sprayed cherries of the "Spanische Weiße Knorpelkirsche" and "Schöne aus Chatenay" varieties were peeled (about $\frac{1}{2}$ mm) with razor-blades. They were cut in such a manner that a strip of equal width was removed, and the blade was kept at the same depth in the fruit in order that there would be as little contact as possible between the poisoned peel and the blade. By using a new blade each time (the strip of peel cut off was about 4 mm wide), none of the preparation was transferred from the peel to the meat of the fruit. Proof of this was provided by cherries immersed in 0.05 % "E 605" conc. which after drying for 30 minutes were prepared in the same manner. Flies placed on the meat of the fruit remained normal for a number of days in each case.

After peeling 4 cherries in each case, the strips separated from the outer layer of the fruit meat were put under a Petri dish and tested with 100 flies. In order to check on the active ingredient which had diffused in the interior of the fruit, the stone was carefully removed. There was sufficient fruit meat clinging to it to feed the flies. There were 4 stones and 100 flies in each Petri dish.

It only took two days to definitely prove by means of preliminary tests with cherries sprayed on June 13 that the active ingredient had penetrated to the stone of the cherry. This finding was confirmed (Table 3). The "Spanische Weiße" variety was sprayed with 0.03 % and 0.05 % "E 605" conc. In the replication carried out on June 27 with the "Schöne aus Chatenay" variety only those cherries treated with 0.05 % "E 605" conc. were examined to confirm the first results. The flies were placed on the cherries 24, 48 and 72 hours after spraying. The cherries had been picked and peeled in the manner described above immediately beforehand. The last three columns of the table contain the average values of 50 % mortality from four replications together with the true mean errors. The active ingredient content, which was again determined according to the given function (Fig. 2) from the time of 50 % mortality, is given in brackets underneath the T 50 % values.

Table 3. Toxicity of several layers of fruit meat, taken from cherries, on *Drosophila melanogaster* M. 1 to 3 days following treatments with "E 605" conc. and the content of diethyl-p-nitrophenyl thiophosphate in mg/kg evaluated from the time of 50 % mortality together with the true mean errors from 4 replications. The active ingredient content is given in brackets.

Date of treatment Variety	"E 605" concentration in %	Fruit meat layer	Times of 50% mortality in minutes and "E 605" active ingredient () expressed in days following the treatment		
			1	2	3
22. 6. Spanische Weiße	0.05	outer layer	1502 ± 64.6 (1)	2500 ± 451 (0.41)	4550 ± 457 (0.16)*
	0.03		2370 ± 87.4 (0.45)	3787 ± 508 (0.22)	5575 ± 585 (0.12)*
	0.05	inner layer	2995 ± 473 (0.31)	3020 ± 250 (0.28)*	6375 ± 704 (0.1)*
	0.03		3892 ± 396 (0.21)	5050 ± 455 (0.13)*	
27. 6. Schöne aus Chatenay	0.05	outer layer		2095 ± 75 (0.32)	4897 ± 560 (0.15)*
	0.05	inner layer		3087 ± 108 (0.21)*	5625 ± 215 (0.12)*

*) = attained mortality of flies between 10 and 50%.

Discussion

Diethyl-p-nitrophenyl thiophosphate penetrated to the stone of the fruit within 24 hours following the application. There is a definite head of concentration between the peel and the outer layers of fruit meat. The toxicity in the fruit meat is at a maximum 24 hours following the treatment, and this decreases to a negligible value after a further 3 days. The maximum content determined following an application of 0.05 % "E 605" conc. was 1 mg/kg for the outer layer of fruit meat, and up to 0.31 mg/kg for the layers around the stone. This means that there is a clear parallelism to the quantities of active ingredient applied. After 4 days the penetrated active ingredient had been inactivated to such an extent that the residues had reached a value too negligible to be checked. It was only in individual cases where a high concentration (0.05 %) had been applied that flies still succumbed. Flies placed on cherries five days following spraying showed no symptoms whatsoever of poisoning. This means that the application of larger quantities of preparation does not considerably increase the longevity of the toxic effect of the fruit meat. Apparently the capacity of the peel for the phosphoric acid esters is slight, corresponding to its thickness. The excess quantity penetrates into the fruit where it is inactivated.

In reality the penetrated quantities of ester should be greater. Since the tests lasted 3 days in individual cases, it is probable that the toxicity recedes

due to the fermentative decomposition in the fruit meat. Furthermore, the translocation of additional quantities of ester from the peel to the fruit was prevented by peeling the cherries.

3. The movement of phosphoric acid ester in the peel of the cherry

Method:

The tips of fruits of a late-ripening large white-heart variety were coated with 0.03 % "E 605" emulsion on June 29. 2 days later the tips and the stalks of these cherries were removed. The tips and stalks of 4 cherries were placed under a Petri dish and tested with flies. The details of the tests are given below:

Test 1: Treatment 29. 6. ("Knorpelkirsche" variety)

Test started 1. 7. (28 hours later)

Average values from 4 replications each with 30 flies (*Drosophilinae*).

Eradication of flies after 39 hours:

a) treated tips 48 ± 5.2 %

b) untreated stalks 48 ± 5.2 % (identical with value a) only by coincidence).

Both parts of the cherry revealed the same toxicity to the flies. Consequently "E 605" had spread evenly over the fruit within 2 days. A replication of the test on the "Spanische Weiße" variety (July 4) produced a similar situation after 3 days (Test 2). The obtained figures for eradication in per cent only deviate slightly.

Test 2: Treatment 4. 7. ("Spanische Weiße" variety)

Test started 7. 7. (74 hours later)

Average values from 6 replications each with 30 flies (*Drosophilinae*).

Eradication of flies after 18 hours:

a) treated tips 29.7 ± 2.5 %

b) untreated stalks 25.5 ± 3.4 %

4. The lasting effect of diethyl-p-nitrophenyl thiophosphate on green and half-ripe cherries

Method:

10 ripe and 10 green, hard cherries were selected on a tree of the "Frühe Französische" variety, and immersed, while still on the tree, in a flask containing 0.1 % "E 605" conc. The adhering quantity of preparation was determined by re-weighing the flask. Altogether 940 mg adhered to the 10 ripe cherries, and 735 mg to the 10 green ones. After allowing them to dry for a short period, and then partially repeating the immersion of the green cherries (5. 6. 51), the total quantity adhering to the fruit amounted to 951 mg.

Almost 4 days later (June 9, 12 a. m.), the ripe and green cherries were picked separately, cut up with a scalpel and placed in a graduated flask filled up to 250 ccs with acetone. Afterwards the pulps were extracted, each for 7 hours, and subsequently the extract was evaporated and the residues with the applied insecticide of a) 46 ccs of green cherries and b) 48.5 ccs of ripe cherries each filled up to 50 ccs with acetone.

Since a preliminary test had shown a clear difference in the content of insecticide, the following dilutions were prepared with an extract obtained in the same manner as above from untreated cherries:

- a) extract from green cherries: 1, $\frac{1}{2}$;
- b) extract from ripe cherries: 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$.

The test was repeated four times, and in each 2 ccs of the above solutions were pipetted onto a round filter (Schleicher-Schüll SS 595, diameter 9 cm). After drying for 16 hours, the filters were rolled together and placed in test tubes each with 100 flies. In addition a minute quantity (enough to cover the tip of a knife blade) of apple sauce was added to each tube. The mortality times together with the mean errors are given in Table 4.

On comparing the obtained values of the two series with each other, it is seen that in the extract from the green cherries four days following the treatment there is only slightly less than half the insecticide contained in the red cherries. When plotted in a graph, the values amount to exactly 47 and 49 %.

The reason for the smaller residue in the green cherries lies in the fact that the fruit has not stopped growing and the enzymes are more active in decomposing the phosphoric acid ester. In addition it should be pointed out that the ripe fruits, or rather their peels, contain more secondary plant substances including fats, wax and wax-like substances (Paech 17).

As far as the practical control of cherry fruit flies is concerned, no great significance is attached to the established difference since the preparation anyway does not have a long lasting effect, a fact strengthened by the results of later tests.

Table 4. Toxicity of extracts from red and green cherries on *Drosophila melanogaster* M. following "E 605" conc. treatment. The figures indicate the 50 % mortality in minutes together with the true mean errors from 4 replications.

Dilution series	T 50 % Extract from red cherries	T 50 % Extract from green cherries
	in minutes	
1	288 ± 9.5	390 ± 33.8
$\frac{1}{2}$	376 ± 12.4	541 ± 28.4
$\frac{1}{4}$	527 ± 13.6	
$\frac{1}{8}$	930 ± 24.5	

5. Conclusion

The experiences made in these tests do not in any way contradict the results given in the literature. They can be supplemented by the paper of Frohberger (8) who carried out similar investigations on other fruits.

The cherry has less lipide coatings than, for example, the apple and the grape. According to Frohberger phosphoric acid ester was traced in the apple peel for 10 weeks and in the grape peel for 18 days, and in fact the toxicity hardly decreased during these periods. The cherry might therefore be

given a placing between the fruits and foliage leaves examined by Frohberger. The resistance put up by the cherry peel against the penetrativity of the insecticide is slight. The rate at which the ester passes into the fruit is particularly striking. After 17 days there was no further trace of it in the cherry peel.

IV. The efficacy of diethyl-p-nitrophenyl thiophosphate against *Rhagoletis cerasi* L.

1. Ovicidal effect

Previously nothing was known of phosphoric acid esters having an ovicidal effect on *Rh. cerasi*. Such an effect was however assumed, this being based on the good results obtained with "E 605" sprays, although it was in opposition to experiences gained in the control of eggs of other insects (Thiem 35, Holz 11, Müller 16, Merckenschlager 15, Speyer 24).

Method:

Wild cherry tree branches bearing fruits heavily colonized with eggs of the cherry fruit fly were sprayed on May 19 with "E 605" conc. 5 branches were used for each concentration, and after removing half the leaves they were kept fresh in water.

The cherries were examined after 14 days with the following result:

"E 605" conc.	Number of cherries	Number of eggs	Number of living larvae
untreated check	154	183	123
0.015 %	113	138	2
0.03 %	99	113	0
0.05 %	112	123	0

Apart from the two living larvae in the weakest concentration, most of the other larvae died after bursting the shell, in fact even before they emerged from it. Undamaged egg shells with larvae not fully developed were a rarity. Since such cases also occurred in the untreated checks — confirming the finding of Thiem (26) that not all eggs develop into young larvae — they are of no significance with respect to the effect of the preparation. A genuine ovicidal effect on the part of phosphoric acid esters has not been established.

No explanation could be given as to whether the emerging larvae are poisoned on the egg shell or whether the fruit meat still contains sufficient ester toxic to the larvae. I give preference to the latter possibility since it is likely that active ingredient from the ester depot in the peel diffuses in the outer fruit layers and kills the larvae.

An embryonal development of 6 to 8 days is to be assumed at the average temperatures of 18 to 22°C in the test room (Wiesmann 37, Thiem 26). The spray must have been effective for the same period, at least on the eggs which were laid shortly before the branches were cut. Many cherry fruit flies were seen on the same day.

2. Effect against eggs and young larvae

Method:

Treated cherries were enveloped on the tree in cellophane-gauze cages at various intervals following the application of "E 605" conc., and colonized with puberal cherry fruit flies (Table 5). The flies were only kept 2 to 3 days in the cages in order to keep a check on the approximate age of the eggs. An examination of the cherries after 10 to 19 days gave the number of larvae still alive. Some of the flies originated from breeds of the Institute, others were caught on *Lonicera* bushes. The flies from the Institute's breeds were at least 12 days old.

The caged flies were able to feed on several leaves coated with highly concentrated sugar water. In the untreated checks there were constant cases of flies dying for no apparent reason. Probably this was due to a lack of dew which provides the moisture necessary for making it easier to take nutrition. This assumption is strengthened by the observation that in dry air and at a temperature of up to 27°C, flies fed with sugar, beet syrup and

Table 5. Egg laying of *Rhagoletis cerasi* L. which were caged on cherry branches of the „Spanische Weiße Knorpelkirsche“ variety, and the number of larvae hatched from these eggs. The trees were treated on June 15, 1951 with 0.03 and 0.05 % "E 605" conc. washes.

"E 605" concentration	Time the flies were caged	No. of flies		No. of living flies	No. of infested cherries	No. of eggs	No. of hatched larvae	Date of investigation
		♀ ♀	♂ ♂	♀ ♀				
0.03 %	17.—19. 6.	13	15	0	3	3	0	
	19.—21. 6.	11	13	5	3	5	3	5. 7.
	21.—23. 6.	15	18	10	14	17	16	
	23.—25. 6.	20	21	18	12	14	14	
	25.—27. 6.	21	25	19	12	16	16	12. 7.
	27.—29. 6.	18	22	18	16	19	17	
	29.— 1. 7.	22	22	20	10	11	10	
	1.— 3. 7.	25	25	21	23	26	24	15. 7.
0.05 %	17.—19. 6.	12	15	0	0	0	0	
	19.—21. 6.	10	14	3	5	6	4	5. 7.
	21.—23. 6.	20	25	14	11	15	10	
	23.—25. 6.	21	24	15	5	18	17	
	25.—27. 6.	19	21	18	9	12	12	12. 7.
	27.—29. 6.	25	30	24	16	20	18	
	29.— 1. 7.	15	20	11	11	21	19	15. 7.
	1.— 3. 7.	23	27	20	13	28	25	
	15.—17. 6.	12	16	10	18	24	22	
	17.—19. 6.	9	12	8	14	15	13	
	19.—22. 6.	8	10	8	13	16	15	4. 7.
	22.—24. 6.	10	14	7	9	18	16	
	24.—27. 6.	6	10	6	7	12	8	
	27.—29. 6.	8	11	7	11	15	14	13. 7.
	29. 6.— 3. 7.	12	15	11	16	19	18	

honey could only be kept alive for 2 to 3 days at the most despite the fact that they were provided with water in jars. On the other hand they lived for several weeks in a room with a constant temperature (20° C) and 90 to 100 % relative humidity.

The test was carried out on a tree of the "Kunzes Kirsche" variety and on another of the "Spanische Weiße" variety (Table 5). Both tests were very similar; therefore no importance was attached to exactitude in the case of "Kunzes Kirsche". The fruit meat of the "Kunzes Kirsche" variety was already slightly soft. The "Spanische Weiße" variety was ready for picking 14 days later.

Discussion:

During the first four days after spraying (up to the 17th and 19th June) all the caged flies died before they laid eggs. In the following two days some more of the flies were killed. Afterwards no further effect against the imagoes was observed. Any mortality during this period was due to natural causes.

There was only one case (Table 5, 0.03 %, "E 605" conc.) in the first colonization test where 3 eggs were observed in which the larvae succumbed when emerging. Eggs laid later led to an infestation by maggots which only in the first two days showed a slight difference between the number of eggs and the hatched larvae as compared with the untreated check.

The results agree with experiences mentioned in Section D III concerning the residual effect of the ester on the fruit peel of cherries. There it was possible to trace the ester by means of flies 15 to 17 days following the treatment. Assuming a development period of 6 to 10 days for the larvae due to the increased temperatures in the cages as opposed to the temperatures outdoors, the first viable larvae ought to have emerged 10 to 17 days following the spray.

3. Effect against old larval stages

Method:

A late-ripening "Knorpelkirsche", approx. 50 % infested by larvae of different sizes was thoroughly sprayed, half with 0.03 % and half with 0.05 % "E 605" conc., so that every cherry was wetted. The treatment was carried out on June 29 at 9 p. m. The diameter of the tree crown was 7 metres. 25 litres of spray wash of each concentration were applied.

Discussion

The test has shown us the following (Table 6): Young larvae (L_1) are far more sensitive than the old larvae (L_n), and therefore die quicker. Following a total of 8 days all the maggots in both tests were dead. The larvae were killed much more quickly with 0.05 % "E 605" conc. than with the 0.03 % preparation. A striking feature was that 8 days following the 0.03 % "E 605" conc. spray, 42 % of the infested fruits — probably due to the irritant effect of the poison — had been deserted by the larvae, this being recognized from freshly bored holes where the larvae had made their exit. These holes were not to be found on cherries treated with 0.05 % "E 605" conc. Obviously the larvae were not able to escape from the fruit in this case as a result of the greater initial effect of the ester set off by the stronger dose. It should also be mentioned that in the cherries treated with 0.03 % "E 605" conc. 33 % of the larvae pupated, and 25 % in those treated with 0.05 %. The pupae were mostly deformed (Fig. 3) and no longer capable of living.

From 31 of these pupae, which were kept on slightly moistened paper in Petri dishes at 20° C, only four appeared normal after 6 weeks, while the other 27 rotted.

In a test carried out by Thiem (35) in the Spring of 1952, "E 605" conc. was only partially effective against old larvae of *Rhagoletis cerasi*. On June 25 one tree each of the weakly infested varieties "Schneiders späte Knorpel", "Butt-



Fig. 3. Pupation of *Rhagoletis cerasi*. Top: normal; bottom: deformed following "E 605" treatment.

Table 6. Effect of "E 605" conc. on the larvae of the cherry fruit fly.

No. of days following the treatment	"E 605" concentration	No. of larvae												Total No. of larvae
		normal				damaged				dead				
		absolute			% L 1-3	absolute			% L 1-3	absolute			% L 1-3	
		L 1	L 2	L 3		L 1	L 2	L 3		L 1	L 2	L 3		
2 1/2	0.05		4	8	19		5	40	70	5		2	11	64
3 1/2	0.03						12	51	46	15	25	34	54	137
	0.05							16	39		5	20	61	41
5	0.03						2	2	11	2	9	7 (16)*	89	38
	0.05					2	7	8	25	2	9	17 (23)*	75	68
8	0.03										31		100	31
	0.05										38		100	38

* = deformed pupae, incapable of living.

L 1 = small larvae, L 2 = medium sized larvae, = L 3 fully grown larvae.

ners rote Knorpel" and "Hedelfinger Riesen" were sprayed with 0.03 % and 0.05 % "E 605" conc. Whereas all the young larvae died, 20 % of the fully grown larvae remained alive. In this test the fruits were noticeably large, and in this connection surpassed those treated the year before when all the larvae were killed.

A parallel spray with 0.05 % "Systox" produced a similar situation after 7 days. Practically 30 % of the old larvae remained alive.

V. Deliberations over the application of diethyl-p-nitrophenyl thiophosphate in practice

1. Absolute residue of phosphoric acid ester in cherries

Cox (12) writes in a report from the U.S.A. of the residues of active ingredient determined in two tests in which cherry trees were treated with "Parathion" (diethyl-p-nitrophenyl thiophosphate). Following two sprays carried out at an interval of 7 days with a concentration corresponding to 0.05 % "E 605" conc., the residue of diethyl-p-nitrophenyl thiophosphate 18 days after the last application amounted to 0.17 mg/kg cherries. In another test (1948) two sprays were carried out at an interval of 14 days, and 0.05 mg/kg cherries was traced 14 days following the last spray. Here the spray concentrations corresponded to 0.023 % "E 605" conc. The residue in both cases was very low.

My own observations are limited to a comparison of the toxic residues on the peel (Table 1). To determine the starting value, cherries just prior to the ripe stage were immersed in 0.03 % "E 605" conc. 6.55 g of wash stuck to 100 cherries. Calculated on the weight of the cherries the pure amount of active ingredient was 2.62 mg/kg. In practice less wash clings to the cherries following a spray. To keep on the safe side, however, the larger quantity of active ingredient will be taken into consideration in this paper.

The fatal dose for an adult human is 448 mg (Hecht and Wirth 10). In order to take a fatal dose, therefore, an adult person would have to eat 171 kg of freshly sprayed cherries.

By studying the results of the tests carried out (Table 1), in which the ester residue was traced in the peel, one can form an approximate picture of the residues to be expected in the days following. After 2 days it can be assumed that the ester applied by means of a spray has either penetrated into the cherry or it has spread in the fruit peel. From the second day onwards the ester content drops until it is $\frac{1}{32}$ of the original value (7) — as traced on the 2nd day — at the final check carried out on the 17th day in the case of "Kunzes Kirsche", $\frac{1}{20}$ on the 15th day with "Spanische Weiße Knorpelkirsche" and $\frac{1}{24}$ on the 15th day for "Schöne aus Chatenay". That conforms to a residue of 0.082, 0.13 and 0.10 mg/kg cherries respectively as based on the above-mentioned quantity of active ingredient which clings to the fruit following a spray. Calculated in the same manner the residue after 8 days is $\frac{1}{15}$ of the applied active ingredient in the one case, and $\frac{1}{6}$ in the other two cases. With a content of active ingredient amounting to 0.1 mg/kg, 4480 kg of cherries are required before the fatal dose is obtained.

2. Date of application and the prospects of success

From the results so far gained the success of a control with the phosphoric acid ester "E 605" can be determined in advance, although limits are set to this prediction by the biological peculiarities of the pest which themselves cannot be foreseen.

A meticulously applied spray with 0.03 to 0.05 % "E 605" not only kills those larvae already present in the cherries but also those which hatch out in the fruit during the following ten days. However, the cherry fruit flies are normally not sufficiently poisoned since the contact insecticidal effect of the ester speedily dies away. The flies are, therefore, able to continue laying eggs which lead to an infestation by maggots.

A suitable spraying date is a factor of decisive importance for success in the field. This should be fixed at ten days prior to the harvest in order to obtain, at all events, cherries which as far as possible are free of maggots since any larvae emerging after the 10th day following a spray are able to survive. For hygienic reasons sprays should be carried out as early as possible, 14 days before the harvest. During this time it is unlikely that there will be a serious infestation since the cherry fruit fly avoids ripe cherries (Thiem 27).

Should the cherries be colonized with eggs four weeks before they ripen, which may occur above all on very late cherries, it is advisable to apply 2 sprays in cases where "E 605" is used exclusively. The first spray is designed to attack the emerging larvae in order to prevent premature destruction of the cherries, while the second spray should be carried out about 10 days later so that the cherries remain hanging for another 14 days until the harvest. In most cases one spray will be sufficient. It is always an advantage to keep a constant check on egg laying because this indicates in advance the approximate date when the first of the young larvae are due to appear. Furthermore this serves to determine whether a second treatment is necessary. A new infestation can be delayed, for example, when the spray is followed by weather which is unfavourable for egg laying.

In confirmation of the aforesaid, the following details of an outdoor treatment are given:

Variety: Late "Knorpelkirsche".

Location: Schriesheim/Bergstraße.

A tree with a crown diameter of 8 metres was treated on June 14, 1951 with 40 litres of 0.03 % "E 605". Equipment: Knapsack sprayer (10 atm.) with malacca cane and fine jets. Infestation of fruits on June 14: larvae 16.5 ± 2.2 %, eggs 17 ± 1.3 %. After 15 days (June 29) the larvae infestation was 2.2 ± 0.9 % as based on 4×100 cherries. The 1—2 mm large larvae which were still alive must have originated from eggs laid after June 14. On 4 untreated trees in a radius of 150 metres, 15 to 41 of each 100 cherries examined were infested with maggots on June 14. The cherry fruit flies seen at the test site on June 29 apparently shied the cherries which were then in an advanced stage of ripeness.

3. Discussion of several control operations

Supplementary to the results of the preceding investigations, it is intended to discuss several spray operations which can be explained consistently from the egg laying investigated. Detailed reports of the sprays carried out under the supervision of Dr. Thiem were placed at my disposal.

The above-mentioned results were finally confirmed by a large-scale test in which "E 605" conc. was applied by means of an atomizer.

A success could only be thought of in all the tests when the cherry fruit flies had laid the majority of their eggs before spraying. It was an accepted fact that eggs laid after spraying caused a new infestation by maggots. From Thiem's reports (35) it is pointed out that the application of "E 605" does not kill the

imagoes present to an adequate degree since the preparation is only fully effective on the leaves as a contact poison for 1 to 2 days at the most. In addition to this *Rhagoletis cerasi* creeps into hiding places on the tree or in the ground in cool and rainy weather. Therefore when a treatment is followed by weather which is unfavourable for the flying activities of the pests, a large number of the flies escape eradication.

Test "Giedensbach 1949" (Thiem 35), Table 7:

The start of the egg laying, in conformity with the temperature, was concentrated on May 28 and 29. In the warm locations, already more than 90 % of the cherries were colonized with eggs by June 2 (No. 13—16) while in the cooler locations the infestation did not start until later. To a certain extent good results were obtained (No. 10—15) even though recent experience has shown that the sprays were carried out too early (June 2). The reason for this is the biological behaviour of the flies in cool weather on the one hand and the degree of ripeness of the cherries on the other hand. At first the temperatures prevailing between the date of the spray and June 6 did not rise above 18° C which excluded any possibility of an increase in infestation during this period. From June 6 to June 8 a further climax was observed with respect to the laying of eggs which explains the later infestation by larvae (tree No. 6—8). A striking feature was that there was no further infestation on No. 10—12 following the control. Apparently in this case *Rh. cerasi* shied the cherries due to the advanced stage of ripeness. The apparent contradiction in the infestation figures as obtained from the continuous observation of one tree is due to the manner in which the checks were taken (100 cherries were examined in each check). To what extent the egg infestation increases on those trees heavily attacked in the early stages (No. 13—16) cannot be clearly seen from the infestation figures since the density of egg laying, i. e. the number of eggs per cherry, which naturally is greater in the heavy infestation, does not appear clearly enough in the infestation expressed in per cent.

The larval infestation was also suppressed to a very great extent on carrying out a combined spray with "DDT" (No. 14—16). The larval attack encountered later may have been due to eggs being laid after spraying.

Test "Giedensbach and Hesselbach (Black Forest 1950)" (Thiem 35):

At both locations egg laying began at the end of May, just as in the previous year, although this time it was not so severe. On the days the treatment was carried out, the infestation on May 30 in Giedensbach amounted to an average of 38 % (4.2 to 60 %) on 11 trees examined, and on June 1 in Hesselbach there was an average of 9 % on 3 trees examined. During the following days the egg infestation continued to increase until an average of 74 % of the cherries were infested by living larvae on June 19 in Giedensbach and 73 % on June 20 in Hesselbach. By the time of the final check at picking time on June 29 and 30, 95 % (Giedensbach) and 67 % of the cherries were infested by maggots. The difference in infestation figures between treated trees and untreated checks was insignificant. Obviously the failure is due to the treatment being carried out too early and the subsequent increase in egg laying. The test again confirms that flies already present cannot be eradicated with "E 605", otherwise egg laying should have been stopped for at least 10 days since freshly hatched females are not ready for laying until after about 10 days (Wiesmann 37), unless of course flies attacking from untreated trees are to be held responsible.

Table 7. Control of *Rhagoletis cerasi* L. in Giedensbach in 1949 (Thiem). Rise in egg and larval infestation by *Rhagoletis cerasi* following sprays with "E 605" f.

No.	Date of treatment	"E 605" f concentration in %	a) egg and b) larval infestation in %									
			Date of investigation									
				2. 6.	9. 6.	13. 6.	17. 6.	19. 6.	20. 6.	22. 6.	24.—26. 6.	
1			a			83	90		95		95	
			b			8	28		30		77	
2			a			75	76		96		97	
			b			9	6		34		91	
3	untreated check		a	38	86	90		89		95	100	
			b	0	0	17		33		61	70	
4			a	52	87	84		97		98	100	
			b	0	0	6		34		58	75	
5			a	31	53	76		84		89	100	
			b	0	0	22		34		55	87	
			Date of investigation									
			27. 5. 28. 5. 1. 6. 7. 6. 9. 6. 13. 6. 17. 6. 20. 6. 24. 6.									
6			a	18		18	15	20	20	37	70	55
			b	0		0	0	0	2	6	15	21
7	2. 6.	0.03	a	3		4	15	58	87	81	99	100
			b	0		0	0	0	0	5	10	63
8			a	9		7	17	59	73	85	92	93
			b	0		0	0	0	5	7	10	31
9			a	6		12	30	60	72	83	20	97
			b	0		0	0	0	3	5	10	50
			Date of investigation									
			2. 6. 7. 6. 10. 6. 13. 6. 18. 6. 21. 6. 24.—26. 6.									
10			a	44	40	30	24	47	49		29	
			b	0	0	2	1	0	2		2	
11	2. 6.	0.05	a	47	47	36	46	51	43		52	
			b	0	0	3	0	7	3		3	
12			a	55	40	26	49	33	48		48	
			b	0	0	0	4	6	4		6	
13			a	99	97	90	98	90	94		97	
			b	0	4	0	5	15	0		20	
			Date of investigation									
			1. 6. 8. 6. 11. 6. 14. 6. 19. 6. 22. 6. 24.—26. 6.									
14		0.05	a	88	91	83	74	76	84		82	
			b	0	2	4	12	10	13		6	
15	2. 6.	"E 605" f and preparation X	a	81	92	81	73	82	87		86	
			b	0	3	8	5	10	18		20	
16		3.0	a	91	90	66	98	99	93		47	
			b	0	3	10	16	22	21		39	

Test "Mühlheim, near Coblenz 1950" (Schmitt 35), Table 8:

Only partial success was gained in this control. Once again the sprays were applied too early (No. 1 to 6). The infestation by maggots must be explained by egg laying following the application of the sprays. Better results could have been expected under Nos. 6 and 7. There must, therefore, have been a sudden and violent outbreak of egg laying following the second spray.

The possibility of that happening is proved by Thiem (26) who observed an infestation of 40 to 47 % in one single day. It also appears likely that some of the living larvae originated from eggs laid before the last treatment. The larvae were still small even at picking time and they were hardly noticed by the fruit growers. The higher concentration of 0.075 % "E 605" conc. did not produce any great increase in the period of effectiveness, a fact already pointed out. However, the strength of the concentration is not a suitable criterion since the absolute quantity which is applied to the cherries is the decisive factor for a successful control. The reports do not give any information either on this point or about the thoroughness of the treatment.

Table 8. Control of *Rhagoletis cerasi* L. with "E 605" conc. in Mühlheim near Coblenz in 1950 (Schmitt). Egg and larval infestation of the cherries in % following the sprays.

No.	Date of treatment	"E 605" conc. Concentration expressed in %	(Egg) and larval infestation Date of investigation	
			26. 6.	27. 6.
1	5. 6. 50	0.06	(96) 50	
2			(93) 40	
3			(95) 58	
4	5. 6. 50	0.075	(96) 50	
5	12. 6. 50			(100) 58
6	5. 6., 12. 6.			(99) 58
7	5. 6., 17. 6.	untreated check		(99) 30
8				(100) 59
9				(100) 62
10				(99) 65

Test "Dettingen, Giedensbach 1951" (Thiem 35), Table 9:

In Dettingen (No. 2—3), the first "E 605" conc. treatment on June 7 was again carried out too early since egg laying had not yet started. The second treatment on June 22 killed practically all larvae emerging up to June 28. On the atomized trees (0.25 % "E 605") 2 % of the fruits were infested by living larvae, and on the sprayed trees (0.05 % "E 605") the cherries were free of maggots. In view of the egg laying established on June 19 (20 % and 16 %), there would have been a severe infestation by maggots were it not for the treatment. The subsequent

the application of phosphoric acid esters to eradicate the larvae of *Rhagoletis cerasi* L., a definite quantity of insecticide must be applied to each cherry, as opposed to "DDT" control which is directed against the motile flies.

A total of 728 cherry trees of different varieties and of varying ages were atomized with 0.33 % "E 605" conc. between June 16 and June 19. Atomizing as compared with a normal spray enjoys a number of advantages. It reduces the cost of the work since only 2 to 6 litres of the highly concentrated wash are used per tree. By producing a strong secondary wind by means of a fan, the leaves are blown upwards so that the cherries hang freely and can be reached better by the mist jet. Losses resulting from dripping wash do not occur. The adhesion of small mist droplets is better on the waxy peel of the cherries. While it is not possible to treat the tops of large trees with the usual types of sprayers, on calm days the tops of the tallest trees can be wetted quite easily with an atomizer.

The team operating the atomizer worked without respirators. No cases were observed of the preparation having an injurious effect.

The time of the treatment was particularly favourable (June 16 to June 19). The greater part of the eggs of *Rh. cerasi* had already hatched, and the larvae which were still small were less resistant to diethyl-p-nitrophenyl thiophosphate. The embryonal development of the eggs not yet hatched was practically completed since the eggs had already been laid before June 9 which was the last day of the "DDT" treatment. The larvae emerged during the days following atomizing at a time when the active ingredient residue was still sufficiently effective to kill them.

50 of the trees treated with "E 650" conc. had not been previously treated with "DDT" (Table 10). The check cherries were taken from trees which had been wetted on all sides. In cases where difficult ground had to be traversed with the result that the trees could only be treated on one side, the success was naturally not so great. Admittedly the fine mist droplets penetrated the foliage wetting all parts of the tree even when applied from only one side, but not enough active ingredient got through to the fruits on the side away from the atomizer. The control met with complete success in all cases where the cherries were thoroughly and directly wetted by the mist jet. For this reason tree tops not wetted so thoroughly were found, on being examined later, to be slightly more infested by maggots than the lower parts of the tree crowns.

In the single checks (Table 13) the trees were marked, and the same number of cherries were removed from around the outside of each, starting from the bottom to about halfway up the crown. The examination was carried out in this manner shortly before the treatment and several days afterwards. The untreated trees on the boundary of the district showed on June 21 to 25 (Nos. 1 to 5) a larval infestation of the cherries totalling on the average 84 %. Egg laying had increased by this time to over 100 %, namely each cherry was populated on the average with more than one egg. The infestation on the trees wetted only with "DDT" (No. 6 to 8) was halted as soon as they were treated so that the average larval infestation (55 %) on June 21 corresponded with the egg infestation ascertained on the day atomizing was carried out. According to this (No. 9 to 20), in face of the egg infestation (June 14 and 17), an average infestation by maggots amounting to 45 % would have to have been reckoned with on the assessed trees on June 14 and 17, had not an after-treatment with "E 605" conc. been carried out. However, the fruits were in reality infested with only 2 % living larvae.

Table 10. Control of *Rhagoletis cerasi* L. with "E 605" conc. and atomizers in Dettingen/Erms in 1952. Infestation of cherries with eggs and larvae before the treatment, and with living larvae after the treatment, both expressed in %. Each average value with the true mean error was obtained by evaluating 5×50 cherries.

Obtained by averaging 3 X 30								
No.	Location	Date of "DDT" treatment	Date of "E 605" conc. treatment	No. of trees examined	No. of eggs and larvae found in % before treatment		No. of living larvae in % following treatment	
					14. 6.	17. 6.	21. 6.	25. 6.
1	South slope	untreated check		3	87 ± 2.1		85 ± 3.1	
2				98 ± 1.9		95 ± 1.4		
3				84 ± 3.0		81 ± 2.3		
4	North slope			3		98 ± 3		91 ± 2.0
5				2	71 ± 4		69 ± 1.6	
6	South slope	9. 6.	not treated	1	51 ± 4.1		48 ± 1.7	
7				59 ± 1.0		56 ± 0.6		
8				67 ± 0.8		62 ± 1.0		
9	South slope	9. 6.	16. 6.	3	44 ± 3.0		4 ± 0.7	
10			16. 6.	2	52 ± 2.1		1 ± 0.2	
11			17. 6.	2	58 ± 0.2		2 ± 0.3	
12			17. 6.	1	21 ± 2.3		0	
13			17. 6.	2	82 ± 2.6		2 ± 0.2	
14			17. 6.	1	10 ± 0.8		1 ± 0.1	
15			17. 6.	2	65 ± 4.1		4 ± 0.5	
16	North slope	6. 6.		3	28 ± 3.2			1 ± 0.2
17				2	47 ± 1.6			3 ± 0.3
18		7. 6.	19. 6.	3		56 ± 2.1		1 ± 0.2
19				4		44 ± 0.2		2 ± 0.3
20				2		34 ± 3.1		3.5 ± 0.4
	North slope	not treated	19. 6.				25. 6.	1. 7.
21				2	91 ± 1.5	1 ± 0.1	2 ± 0.1	
22				5	72 ± 2.1	3 ± 0.3	4 ± 0.0	
23				4	81 ± 2.4	2 ± 0.3	3 ± 0.4	
24	North slope	not treated	19. 6.	3		83 ± 1.0	0	0
25			(double quantity)	2		78 ± 0.9	0	0

Before the treatment an average of 81 % of the cherries were infested on the trees (No. 21 to 23) wetted for the first time on June 19 with 2 to 4 litres of 0.33 % "E 605" conc. 6 days later (June 25), living larvae were still to be found on the average in 2 % of the cherries. At the next check on July 1, the maggot infestation had increased slightly to 3 % since a number of the late hatching larvae were not killed due to the rapidly receding aftereffect of the preparation. Therefore those larvae hatching out of eggs laid shortly before the preparation is applied have the best prospects of surviving a control with phosphoric acid esters.

The trees of No. 24 and 25 were wetted with 4 to 6 litres of 0.33 % "E 605" conc., in other words almost the double quantity, with 100 % success. A careful examination of the cherries confirmed that diethyl-p-nitrophenyl thiophosphate does not have an ovicidal effect. The larvae do not succumb until the moment they leave the egg shell, or shortly afterwards.

VI. The efficacy of the phosphoric acid esters "Systox" and "OMPA" against *Rhagoletis cerasi* L.

It would be most desirable to have an agent possessing a long lasting after-effect against the eggs of *Rh. cerasi*. In view of this Thiem (35) carried out tests with "Systox" and "OMPA" which are effective for a week against sucking insects.

According to the results of spray tests carried out on *Lonicera* in 1951 and 1952, "Systox" and "OMPA" are suited for the eradication of eggs and larvae occurring in the fruits. The flies themselves are not adequately controlled since the contact insecticidal effect of the agents does not last long enough. In one test 0.1 % "Systox" and 0.3 % and 0.5 % "OMPA" had an after-effect lasting 20 days against the eggs of the cherry fruit fly. This lasting effect of the agents, as opposed to "E 605", only occurs with high concentrations. No difference in the period of effectiveness of the individual agents was determined in the tests carried out on fly honeysuckle (Table 11) on June 20, 1952 (Wiesloch) and on June 24 (Heidelberg) with sprays of 0.05 % "E 605" conc. and 0.05 % "Systox" (0.025 % pure active ingredient), and 0.15 % "OMPA" (0.099 % pure active ingredient). In these tests 500 single fruits were taken from each treated bush on the 12th and 13th day following the sprays and placed in boxes of sand. From the number of pupae counted after 4 weeks, the percentage of pupae can be determined which originated from the infested berries. Since eggs were also laid between the dates of the sprays and the date the berries were picked, the numbers of pupae give an indication of the lasting effect of the individual preparations. All three agents were equally effective in the given concentrations. It was merely in the case of 0.03 % "E 605" conc. that twice as many larvae pupated as in the case of 0.05 % "E 605", "Systox" and "OMPA".

When high concentrations are used there is the danger that toxic residues which are too high are left in the harvest, since it is only with extreme difficulty that it is possible to prescribe the right dose of toxic phosphoric acid ester to be used in the spray with the aim of producing a residual effect against eggs and larvae lasting up to a few days before the harvest. More thorough investigations will have to be carried out to determine whether these agents can be used together with contact insecticides possessing a longer residual effect which at the same time eradicate those flies already present — for treatments of large areas the last spray is carried out approximately 3 to 4 weeks before the harvest.

Table 11. Sprays applied to fly honeysuckle with phosphoric acid esters to control the eggs and larvae of *Rhagoletis cerasi* L. in 1952 (Thiem).

No.	Place and date of treatment	Preparation	Concentration in %	Content of active ingredient in %	No. of eggs and larvae 13 days following treatment in %	Pupae from 500 berries	Per cent of pupae from the infested berries
1	Wiesloch 20. 6.	untreated check			88	533*	63
2		"E 605" conc.	0.03	0.015	96	135	28
3			0.05	0.025	83	57	14
4		"OMPA"	0.15	0.099	76	48	13
5		"Systox"	0.05	0.025	82	65	16
6	Heidelberg 24. 6.	untreated check			78	145	37
7		"E 605" conc.	0.05	0.025	58	26	9
8		"Systox"	0.05	0.025	37	8	4

* from 1000 berries

VII. The combined application of phosphoric acid esters with agents containing "DDT" (dichlorodiphenyl trichloroethane)

The obvious solution to the problem of killing the flies and their various stages of development in the cherries in one operation would be to combine phosphoric acid esters with "DDT".

In 1949 Thiem (35) carried out the first large-scale test with a combination of "E 605" and "DDT" (Table 7, No. 14—16). The larval infestation expected as a result of the large number of eggs laid before the treatment was suppressed to a considerable degree. The infestation by maggots ascertained on June 24 was on the average higher than on trees which were only treated with "E 605". According to this one might assume that this was due to an unfavourable influence of "DDT" on "E 605". However, in view of the different state of the trees, a comparison can only be made with tree No. 13 which was also severely attacked early on. Therefore no differences result from the evaluation when the larger deviations are taken into account. The last larval attack observed (June 24) obviously was partly due to eggs being laid after the control had been carried out. The rain which fell on the days following the treatment reduced the effect of "DDT". The figures for the infestation expressed in % do not give a satisfactory indication of the absolute number of eggs laid. In the case of the high degree of infestation (80—90 %), 5 to 10 eggs must have been laid on every 100 cherries for the total infestation to go up by 1 %.

Vogel (36) reports on good results obtained in Switzerland following the application of 0.15 % preparation Y, an agent containing 20 % diethyl-p-nitrophenyl thiophosphate, combined with a 0.2 % "DDT" preparation (containing 50 % "DDT"). In one example, the larval infestation 28 days after the two agents were applied amounted to 2 % as compared with 52 % on the untreated check. Vogel (36) writes that the laid eggs and the young larvae were killed.

These experiences suggest that the simultaneous application of phosphoric acid esters with "DDT" agents produces good results. Both the spraying process and the atomizing process are suited for such treatments provided they are carried out thoroughly. A further detailed investigation will have to be carried

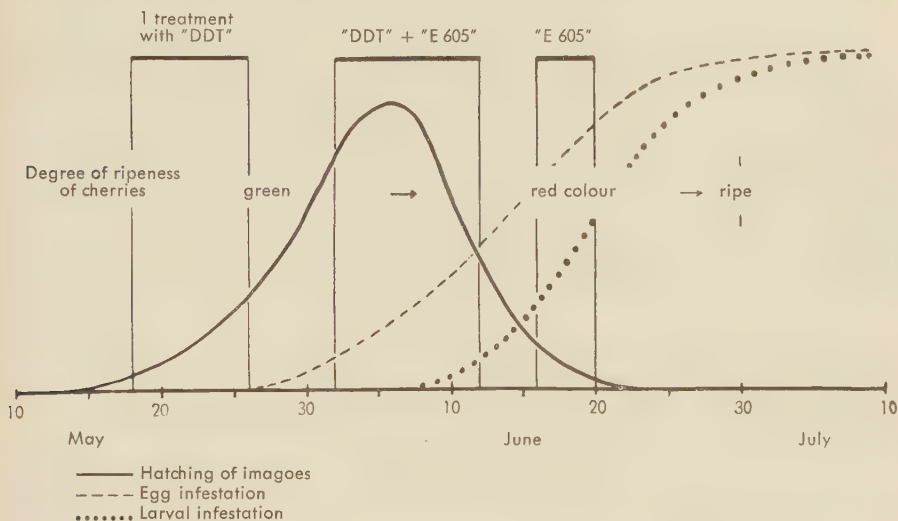


Fig. 4. Periods for the control of *Rhagoletis cerasi* L. with "DDT", "DDT" + "E 605" and "E 605" taking into consideration the biology of the pest and the time the cherries ripen. Biological observations from 1943 to 1952 (Thiem 35).

out to show how far processes in which single trees are wetted are suited. It is, however, essential that in each case a definite amount of ester is applied to every fruit. A disadvantage of this is the high expenditure incurred by using double the quantity of preparation. This increase in costs, however, only exists in comparison with control methods in which successes are achieved through a single treatment with "DDT" preparations.

When the control is carried out on the right date (Fig. 4), at least one spray can be saved as compared with the "DDT" sprays which normally are necessary. Moreover, by using "DDT" and phosphoric acid esters at the same time, difficulties can be surmounted which in field work are caused by a shortage of equipment, bad weather, shortage of labour, and which prevent the first control being carried out at the right time. Also when these preparations are applied simultaneously in the treatment of large areas the greatest success can be expected. The nearer treatments are carried out to harvest time, the earlier they can be stopped. However, the combined spray should not be carried out later than 2 to 3 weeks before harvest since there is the danger of the cherries

becoming too heavily soiled. Fig. 4 gives the dates for single treatments of a late cherry variety taking into consideration the biology of the pest. The biological data are derived from average values obtained at Heidelberg over a period of 10 years (1943—1952). They were compiled from observations made by Thiem (35) on *Lonicera*.

E. The effect of phosphoric acid esters on *Laspeyresia (Grapholita) funebrana* Tr.

I. Problems in the control of *Laspeyresia funebrana* Tr., and its significance

Problems resembling those encountered in the control of the cherry fruit fly are also met with when combatting the plum moth. The winged stage of the two generations lasts several weeks, that of the first generation from May to June, and that of the more harmful second generation from July to August. Although the moths of the two generations are on the wing at different times, the development times of the two larval generations may overlap. The control of the first generation is not so urgent since the damage it inflicts is usually slight. The prevention of an occurrence of the second generation by radically eradicating the first generation could only be effected with measures taken over a large area since there is always the possibility of the pests attacking from adjoining, untreated areas thus threatening the success of the control. According to Fischer (7), isolated orchards are supposed to have been practically rid of the pest for a long period following a successful control. Thiem (35) observed that the degree of infestation in South West Germany fluctuated greatly from year to year. In individual cases it rose to 80 % on damsons, and where orchards had been neglected it often amounted to 50 %.

The imagoes of the plum moth are more difficult to control than those of the cherry fruit fly because, according to Bovey (3), in warm weather they can start laying eggs 2 to 3 days after hatching without taking nutrition. The possibility of the moth coming into contact with contact insecticides is therefore very slight particularly as it only flies at dusk. Probably this was why good results could not be obtained with "DDT" agents. It is also difficult in most cases to control the caterpillars because the path they take from the egg to the point where they bore into the fruit is apparently too short for them to be poisoned. Since the larvae scrape off the harder fruit peels without eating them, they can hardly be controlled with stomach poisons. Greater preference is given to nicotine because it acts on the eggs. The eggs do, however, develop completely although the young caterpillars die the moment they leave the egg shell (Bovey 3). In most cases it is necessary to repeat the treatment several times.

Repeated sprays could be saved when an agent which also destroys the young caterpillars in the fruits is used. Thiem (35) and Fischer (7) pointed out that this is effected by "E 605". In 1946 Thiem was successful in keeping the larval infestation down to 12 %, as compared with the untreated check showing 48 %, by applying 0.05 % "E 605" f. Fischer too was most successful with "E 605" when his results are compared with those obtained from the application of nicotine soap, "Nikopren", preparation H and "Bladan". Following two applications of 0.02 % "E 605" f, the percentage of "wormy" fruit was 7 % as compared to 81 % for the untreated check. Based on his findings, he suggests that the first "E 605" spray should be applied after the first eggs have been laid.

The aim of the following investigations was to probe more closely into the effect of "E 605" so as to be able to use it in field work with the desired degree of certainty. "E 605" was selected because, on the grounds of published test results, it probably has a favourable effect and further because it does not leave behind any troublesome toxic residues in later applications.

II. Behaviour of "E 605" conc. (diethyl-p-nitrophenyl thiophosphate) on the fruit and the leaves of the damson tree

1. Period of effectiveness of phosphoric acid esters on the peel of damsons

Damsons were treated on the tree with 0.03 % "E 605" conc. applied as uniformly as possible by means of a 3 litre hand sprayer. During the following days the fruit peel was removed from the damsons to a thickness of approximately 1 mm with the scalpel, and tested by placing *Drosophila* flies on it. Each Petri dish contained the peel parts from 8 half damsons, and 50 flies. Since the flies could not feed on the green fruit meat adhering to the peel, they had to be given a small piece of apple. The T 50 % values again proved most useful in drawing comparisons. These times ascertained graphically according to the Probit method are compiled in Table 12 together with the mean errors from four replications. It appeared sufficient to give the times in hours, which also simplified the calculation. The results of sprays carried out on July 18, July 28 and September 20 are given.

Table 12. Residual effect of 0.03 % "E 605" conc. on the peel of damsons in different stages of ripeness against *Drosophila melanogaster* M. 50 % mortality is expressed in hours together with the true mean errors from 4 replications.

No. of days following the treatment	Date of sprays and stage of ripeness		
	18. 7. 51 green and hard	28. 7. 51 first appearance of colouring	20. 9. 51 first fruits ripe
1	10.25 ± 0.33	14.25 ± 0.92	8.12 ± 0.25
2	24.8 ± 0.75	23.62 ± 1.60	
3	60.8 ± 2.99	49.40 ± 1.40	39.5 ± 2.1
4	90.5 ± 2.40	70.0 ± 1.40	59.8 ± 2.71
5		89.5 ± 4.42	88.75 ± 3.64
6		94.2 ± 2.66	
8			91.0 ± 4.38

A contact effect of "E 605" on the damsons could not be established with *Drosophila* flies 4 to 8 days after the sprays.

The longer period of effectiveness in the replications is due to the advanced stage of ripeness. While they are still growing, the damsons secrete waxy substances on the cuticle. If the very thin coating is peeled off, it will be seen that it is replaced within a few days, although on ripe fruits a new formation does not occur. Paech (17) also writes that plant parts renew their wax coating as long as the plant is in growth. The epidermal secretions which are also stored in the outer membranes of the epidermis cells enlarged the solvent depot for

"E 605". This explained the longer period of effectiveness, which was equivalent to that in the tests on cherries. It had, however, no connection with the recordings of the relative humidity made at the same location, although this might be conceivable since Paech (17) writes that high relative humidity suppresses the secretion of wax.

Like the humidity, the temperature must also have been of secondary importance. The temperature prevailing on the days following July 18 (1st test series) was 18 to 22° C, below that of the second test series which was 22 to 26° C. According to this, there should be a deviation not conforming with the results.

No difference in respect of the reported extension of the period of efficacy was established in the effect on the eggs and caterpillars of the pest, as will be seen from the tests described later.

2. Penetration of phosphoric acid ester into the fruit of damsons

No information had come to light regarding tests having been carried out to determine the penetrativity of phosphoric acid esters into damsons. Yet considering what was established in the case of cherries, it was quite probable that the esters penetrated damsons.

Test 1:

Damsons were peeled approximately 1 mm thick at different intervals following the treatment, and flies were placed on the outer fruit layers immediately under the peel. The fruit meat from 4 damsons was placed in a Petri dish. No active ingredient whatsoever was found in the outer fruit layers 1 to 3 days after the application of 0.03 and 0.1 % "E 605" conc. All the flies remained normal after being tested for 2 to 3 days.

Test 2:

Extracts prepared with acetone were evaporated in order to trace any small residue of active ingredient. The spray was carried out on August 13, 1951, with 0.03 % "E 605".

- Varieties: a) House damsons, green and hard
b) Nancy Mirabelle, ripe for picking.

The peel was carefully removed to a thickness of 1 mm from 20 damsons at different intervals following the application of the spray. The precautions taken in doing this were the same as those mentioned for peeling the cherries (p. 243). Afterwards 25 g of fruit layers, 2 mm thick, were placed in a beaker together with 500 ccs of acetone. The beakers were left standing 20—24 days for the purpose of extraction, and were shaken daily. Subsequently 20 ccs of this solution were pipetted onto a round filter (SS 595, 9 cm in diameter) placed in one half of a Petri dish. After drying the solutions 24 hours in front of a fan, 50 flies were inserted in each dish. The extraction of the mirabelles was carried out the same way. Analogously prepared extracts of damsons and mirabelles were used as a check. These fruits were immersed in a 0.03 % "E 605" wash 30 minutes before they were prepared to find out whether the active ingredient had been transferred from the peel to the meat of the fruit while they were being peeled.

The extractions were started 31, 49 and 101 hours after spraying. All the flies on the damson extracts prepared in 4 replications were still normal after

49 hours. This meant there was no trace of ester in the fruit meat of the damsons. The insecticide in the mirabelles was found to be still present in very slight concentrations 31 and 49 hours following spraying (mortality of flies $10 \pm 2.2\%$ and $14 \pm 2.7\%$). This deviation is explained by the advanced degree of ripeness of the mirabelles. The fruits were already soft and had stopped growing.

3. Propagation of phosphoric acid ester on the fruit peel of damsons

The propagation of the agent on the fruit peel of damsons may be of significance for the practical effect against the pests, and was therefore subjected to a close investigation.

It was not possible to wet the damsons on one side only since the droplets of the agent flowed together on the waxy peel and did not stick very well. Therefore small pieces of cellulose, the size of a farthing, were immersed in 0.03 % "E 605" conc. and stuck to the tips of the damsons on the tree (27.7). The cellulose pieces continued to stick well even after they had dried. After 7, 24, 48 and 98 hours, 8 fruits were picked at a time, their wetted halves separated from the untreated part by a 2 mm border strip, and the peel parts of every two damsons tested in a Petri dish with 30 *Drosophila* flies.

Only a few flies died on the peel parts of the untreated halves (0.2 to 0.5 %), whereas 50 % of the flies placed on the treated parts 96 hours following the application of the agent died within an average of 30 hours.

According to this a translocation of the ester on the peel cannot be reckoned with to any great extent. There was no reason for repeating the test over a period of 4 days since the effect of the agent will have died off considerably within this space of time.

The test was repeated in a modified manner (October 11, 1951). The stalkless halves of ripe damsons, marked with coloured wax pencil, were immersed in 0.1 % "E 605" in the laboratory. After four days, the treated halves were separated from the untreated peel parts although a 2 mm wide border strip was not applied in order to be sure that the wetted parts did not come into contact with the untreated peel parts. Two Petri dishes were each charged with the peel parts of 4 damson halves and tested with 100 flies.

The mortality of flies in the two Petri dishes containing the treated fruit halves amounted to 100 % after 24 hours, on the untreated halves to 1 % in one dish and to 3 % in the other. These values did not increase later.

This test also confirms that "E 605" only spreads to an insignificant degree on the fruit peel of damsons.

This finding which does not conform with that on cherries is due in my opinion either to the ester being insufficiently capable of penetrating the fruit meat or to its rapid inactivation, with the result that a part is played merely by the translocation in the peel.

4. Absolute phosphoric acid ester residues

It is quite understandable that there is concern about the application of "E 605" shortly before the fruit harvest, for hygienic reasons. However, many investigations have shown that after a few days the ester only leaves a very small residue (Hoskins 12, Ginsbury 9). Hoskins found only slight traces of active ingredient in plums a few days following a "Parathion" appli-

cation. Residues amounting to 1 mg/kg were traceable solely in grapefruits and olives. These disappeared very soon afterwards. Time and temperature are named as being the chief factors determining this.

In view of the finding that the active ingredient remains in ripe fruits somewhat longer, ripe damsons were treated once again late in the season and the absolute residues in them were tested biologically.

Method:

Several extracts were prepared from the peels of the fruits 6 hours to 9 days after they had been thoroughly wetted (August 28) with 0.03 % "E 605" conc. An extraction of the fruit meat was superfluous since "E 605" could not be traced there. On the average 14 damsons were peeled about 1 mm thick in order to obtain 25 g of peel to which 50 ccs of ethanol were added. A note was made of the weight of the damsons required for obtaining the peel. The extracts were left standing in 100 cc flasks until the test was started (December 13). They were shaken daily.

In order to fix a standard for the reliability of an extraction, 4 extracts were prepared after four days from 4×25 g peels in the described method. The individual values were obtained again from 4 replications in the biological test. The values (Table 13) deviated so slightly that the following extracts could be evaluated without a repetition.

By depicting a series of comparative tests (Table 14) carried out on September 3 with given quantities of "E 605", the absolute residues in the peel could be ascertained from the T 50 % values thus obtained (Table 13). In addition untreated damsons were peeled in the same manner, and 25 g of peel placed in flasks with 49 ccs ethanol and 1 cc of an "E 605"-ethanol solution of the given concentrations (Col. 2). These flasks were also left standing until the test was started (December 13).

Table 13. Absolute residues of diethyl-p-nitrophenyl thiophosphate in the peels of damsons following a treatment with 0.03 % "E 605" conc., calculated from the toxicity of peel extracts to *Drosophila melanogaster* M. (Table 14).

No.	Start of extract following the treatment, expressed in days	T 50% expressed in minutes with the true mean errors	mg of active ingredient in 25 g peel	Weight of peeled fruits in g	Active ingredient residue in mg/kg
1	1/4	238 ± 6	0.24	176	1.36
2	1	251 ± 3.25 *	0.23	171	1.34
3	2	266 ± 5.8	0.21	180	1.17
4	3	278 ± 7.8	0.195	173	1.13
5	4	390 ± 18	0.135	182	0.74
6	5	425 ± 9.8	0.125	174	0.72
7	6	526 ± 13	0.124	176	0.705
8	13	843 ± 20	0.065	172	0.38
9	19	1458 ± 72	0.044	171	0.26

* This value was obtained from 4 single extracts each with 4 repeated tests.

The solutions could not be pipetted direct onto glass since the sugar dissolved in them leaves a sticky residue behind which cannot be tested with *Drosophila* flies. Therefore 5 ccs of solution were transferred from each flask in four replications to a round filter (SS 595, 9 cm diameter) placed in a Petri dish. The solutions were dried for 18 hours at room temperature. 100 anesthetized flies were placed on each half peel covered by a glass plate. The mortality was constantly recorded and from this the time in which 50 % of the flies died was calculated according to the Probit method (Col. 4). The values for the last two concentrations were determined by extrapolation since it was only possible to obtain a partial mortality in that particular test (Col. 5).

By means of a graphic representation of Table 14 the corresponding active ingredient residues can be read off from the values of 50 % mortality (Table 13). The residues given in Col. 6 for 1 kg damsons are calculated on the weight of the fruit which was necessary to obtain 25 g of peel. The residues are so slight that they present no danger whatsoever to consumers (fatal dose for fully-grown humans 448 mg active ingredient).

Table 14. Toxicity of extracts from damson peels (containing given quantities of diethyl-p-nitrophenyl thiophosphate ["E 605"]) to *Drosophila melanogaster* M.

No.	Conc. of 1 cc E-solution added	Active ingredient in mg in 25 g peel	T 50% in minutes	Obtained mortality in %
1	1%	5.0	54.2 ± 2.9	100
2	0.1%	0.5	125 ± 4.6	100
3	0.05%	0.25	228 ± 11.1	100
4	0.01%	0.05	1205 ± 136	15
5	0.005%	0.025	4400 ± 511	4

The greater percentage of lipid in the thicker damson peels, and in which the ester dissolved, can be held responsible for the slower rate at which the ester residue disappears in damsons, as compared with cherries. A part of the ester evaporates while another part probably diffuses in the meat of the fruit where it is rapidly decomposed.

High residues should hardly occur in the application of the agent in field work since fully ripe fruits are not subjected to further treatments so late on in the season. "E 605" is rapidly inactivated in fruits which are still in growth. This was mentioned in another part of the paper.

5. Period of effectiveness of phosphoric acid ester on the leaves

Following a treatment with 30 litres of 0.03 % "E 605" conc. (August 24), leaves were removed at different intervals from a well developed house damson and tested immediately with *Drosophila*. Glass tubes (3.5 cm diameter, 15 cm long, and sealed with cellulose) were each filled with 5 leaves of equal size and 50 flies one, two and four days after the treatment (Table 15).

The tests reveal that a contact effect of the ester on the pest can be reckoned with for 2 to 3 days at the most.

Table 15. Average values of 50 % mortality with the true mean errors from 4×50 *Drosophila melanogaster* M. which, following a spray of 0.03 % "E 605" conc. applied to damson leaves, were placed on each of 5 leaves.

Start of test expressed in hours following the treatment	T 50 % in hours
31	11.9 $\pm$ 0.24
42	27.6 $\pm$ 1.5
96	(4 $\pm$ 0.8) *

* = % of dead flies after 60 hours of testing.

III. Effect of diethyl-p-nitrophenyl thiophosphate against *Laspeyresia (Grapholita) funebrana* Tr.

1. Ovicidal effect

An ovicidal effect by "E 605" on *Laspeyresia funebrana* was disputed by Fischer (7). Further details supported by results are not given.

Test 1 (Table 16):

House damsons colonized with eggs of the pest were thoroughly sprayed on July 18 with 0.03 % "E 605". The eggs were in the initial stage of development. After the spray had dried on, 29 damsons with a total of 32 eggs were removed from the treated trees and placed in water by their stalks in a thermo-constant room at 20° C and 95 % relative humidity.

While viable larvae emerged from only 6 % of the eggs in the test, the total in the untreated check was about 90 %. On taking into consideration the time taken for the eggs to develop, which amounted to 7 days at 20° C (Bovey 3), it was apparently only those caterpillars emerging from eggs laid just previous to the treatment which bored into the fruit.

Table 16. Effect of 0.03 % "E 605" conc. on eggs of *Laspeyresia funebrana* Tr. occurring on house damsons.

	No. of eggs used in test	No. of caterpillars boring into the fruit on the days following the treatment				Remarks
		2nd day	4th day	6th day	12th day	
Untreated check	12	3	6	8	11	10 living larvae, 1 egg not developed
Treated on July 18 with 0.03 % "E 605"	32	0	0	2	2	23 eggs fully developed, subsequently died. 6 larvae died after emerging

Test 2 (Table 17):

To test the residual effect of "E 605", fruits colonized with eggs were again taken from the same trees 1 day and 8 days following the treatment, and kept in a thermoconstant room in the same manner as described in Test 1. The total

number of emerged caterpillars was ascertained after a lapse of several days. The eggs were laid before and after spraying. The first caterpillars emerged 12 days following the treatment. Taking into consideration the temperatures prevailing outdoors, it was probable that the development lasted 9 to 10 days. This would mean that at least some of the eggs laid after spraying are able to develop.

Table 17. Residual effect of 0.03 % "E 605" conc. on house damsons against eggs of *Laspeyresia funebrana* Tr., some of which were laid after the application of the insecticide. The treatment was carried out on July 18, 1951.

Date of picking = transfer to test room	No. of eggs used in test	No. of larvae which bored into fruits on the days following the treatment							Remarks
		8	9	10	12	13	17	20	
26. 7. 51 untreated check	22	4	5	5	9	?	20		2 eggs not developed
26. 7. 51 (8 days) following treatment	16	0	0	0	2	2	4	4	12 developed larvae dead un- der the skin of the eggs.

Test 3 (Table 18):

This test was carried out with the intention of clarifying whether the results obtained in Tests 1 and 2 can be applied to outdoor conditions. A house damson was thoroughly treated on July 23 with 0.03 % "E 605" conc. On the days following the application those fruit growing around the tree crown which were freshly populated with eggs were marked with dabs of paint. After a further 12 to 15 days the caterpillars which had bored into the fruits were counted.

Table 18. Residual effect of 0.03 % "E 605" conc. against eggs of *Laspeyresia funebrana* Tr. occurring on damsons. The eggs were laid at established intervals following the treatment (July 23, 1951).

	Time of egg laying in days following the spraying						
	before the treatment	1	2	3	4	5	7
No. of marked eggs	12	3	1	2	8	10	12
No. of larvae boring into the fruits 12 to 15 days following egg laying	3	2	1	1	7	9	10

Due to the low temperature only a few eggs were found during the three days following the treatment, although afterwards the egg infestation increased as the temperature rose.

The table shows that practically all the eggs laid following the treatment hatched out to cause a larval infestation of the fruits. The differences between the number of eggs and the number of larvae are insignificant since not all the eggs on the untreated damsons developed.

Test 4:

On May 18, 1952, 100 "Lützelsachsener" early damsons which had been freshly colonized with eggs of the first plum moth generation were thoroughly sprayed with 0.03 % "E 605" conc., and placed in quartz sand by their stalks. Storage took place in a well-aired greenhouse at temperatures equivalent to those prevailing outdoors.

After fourteen days, 66 larvae had hatched out of 98 untreated eggs which had also been stored in the manner described above, and had bored their way into the fruits. 14 of the 100 treated eggs hatched out, and the emerging larvae started boring paths through the fruits to obtain nutrition. Of the remaining eggs, 51 % had developed almost completely but they died shortly before hatching. The other 34 % hatched out, and the caterpillars died either immediately on leaving the egg shells or on boring into the fruits.

All the tests unanimously show that "E 605" conc. does not possess an ovicidal effect in the actual sense of the word since the eggs of *Laspeyresia funebrana* Tr. complete their development despite treatment with this ester. It is the young caterpillars which first succumb to the extent of 87 % while still in the egg shell or shortly after emerging. Eggs laid after the application of 0.03 % "E 605" conc. hatch out into viable caterpillars. Obviously the ester is dissolved in the egg shell and the young larvae are poisoned on attempting to emerge.

2. Larvicidal effect

Since nothing is known regarding the efficacy of phosphoric acid esters against the caterpillars of the plum moth, sprays of 0.03 % "E 605" conc. were applied to house damsons with a view to establishing whether this ester has a larvicidal effect (July 18, August 1, August 13, August 23).

The results obtained from the treatments were so alike that it was possible to compile them together in one table (Table 19). The infested fruits were evaluated 4 days following each spray, since no further damage was established after this time.

A striking feature is the percentage of originally infested fruits (29 %) which were deserted by the first four caterpillar stages. It is assumed that this was due to the irritant effect of the agent. On the untreated trees only single cases were found of empty larval passages which had not been bored through to the stone. In the test treatments the last larval stage remained unharmed and during its course the caterpillar penetrated through to the stone. This last stage can be easily recognized by its pink, flesh-coloured back and the bright pink of the abdomen. In the first four stages, however, the caterpillars are white on both sides.

An increase of the "E 605" concentration to 0.05 % (August 13) did not improve the effect against the last larval stage. It is, therefore, advisable to use the lower concentration (0.03 %) and to treat more thoroughly. On the average 88 % of the first four larval stages were eradicated by sprays with 0.03 %

"E 605" conc. When the damsons deserted by the caterpillars are included in the calculation, and the larvae in the fifth stage are excluded, the result of the treatment increases to 93 %.

Table 19. Effect of 0.03 % "E 605" conc. on different larval stages of *Laspeyresia funebrana* Tr. occurring in damsons.

		Absolute No.	% of infested fruit
Fruits with deserted passages		101	29
Larvae of stages 1 to 4	dead	154	44
	clear signs of injury (weak movements)	13	4
	normal appearance	7	2
Larvae of stage 5	dead	2	1
	alive	72	20
Total		349	

3. Mode of action against the caterpillars

In view of the fact that a penetration of "E 605" could not be established in the biological test, and whereas caterpillars which had already penetrated the fruit were eradicated, it appeared advisable to look for the penetrative effect of the ester in the vapour phase. The following tests largely support this supposition.

Driggers (5) arrived at the conclusion that "Parathion" produced a vapour action when applied against the caterpillars of the apple codling moth (*Carpocapsa pomonella* L.). Young caterpillars which had penetrated into the fruit were killed when the apples were only treated on the side opposite to the passage bored by the pest. Smith (23) points out that in general the penetrative effect of "Parathion" is due to a local vapour action.

Test 1:

1 cc of "E 605" conc. was pipetted onto a filter paper (7 cm diameter) which lay on the bottom of a specimen flask (12 cm diameter, 18 cm high) under a wire frame 2 cm high. Above this 17 fruits colonized with caterpillars were placed on 5 untreated filter papers (14 cm diameter). The flask was sealed with a ground lid.

After 40 hours at room temperature all the larvae had died (9 L_2 , 1 L_3 , 2 L_5). Since there was no possibility whatsoever of the poison coming into direct contact with the damsons, and also in view of the fact that all 9 caterpillars in a corresponding untreated check flask were normal, it must be assumed that the treated caterpillars died as the result of a vapour effect.

Test 2 (August 22, 1951)

Infested damsons were pierced under water by means of pointed glass tubes into which air had been blown, in such a manner that the tips of the glass tubes came to rest either in the passages bored by the pests or in their immediate

vicinity. The glass tubes, connected to an electric aquarium pump, were secured to the green damsons with beeswax. The arrangement of the fruits, which afterwards were treated on all sides with 0.1 % "E 605" conc., was carried out in the laboratory at 22 to 24° C. For the purpose of drawing a comparison, infested damsons were also pierced with glass tubes and treated but were not connected to the pump. After 44 hours the 6 caterpillars found in 8 aired damsons were still living. Five were in stages 3 and 4, and one in stage 2. Only the caterpillar in stage 2 showed signs of slight injury in the form of weak movements.

Altogether 13 larvae of stages 2 to 4 were found in the 16 fruits which were not aired. 11 of these were dead, one seriously injured, and the other slightly injured. By airing the damsons from the inside it was possible to neutralize the effect of a treatment with 0.1 % "E 605" conc. A repetition carried out on August 24 confirmed the result.

Result of the check after 3 days:

1. Aired damsons: L_2 to L_4 = 6 normal, 1 dead
 L_5 = 1 normal
2. Damsons not aired: L_2 to L_4 = 10 dead
 L_5 = 1 normal, 1 injured, 1 dead.

Test 3:

In conjunction with the last tests carried out, the influence of strong air movement on the efficacy of an "E 605" treatment was closely investigated. It is conceivable that the wind impairs the efficacy of the control measure since the eradication of the caterpillars is obviously due to a vapour action.

On August 18, infested damsons were thoroughly wetted from all sides with 0.03 % "E 605" conc. Two open cardboard boxes (50 × 50 cm, depth 4 cm) were each filled with 50 of the treated damsons. One box was placed in a calm corner of the test room, and the other in front of a fan which produced a wind blowing over the box at a velocity of 1.6 metre per second. The temperature in the test room was between 20 and 24° C. Only the first four larval stages were examined after 46 hours since the last stage is resistant. Only 6 of the 35 caterpillars in the aired damsons were dead (17 %) while of the 31 caterpillars in the damsons not exposed to the wind 26 had succumbed (84 %). If the experiment had been continued the mortality would probably have increased. None of the caterpillars were harmed in 40 untreated damsons, half of which were placed with the wind-exposed fruits and the other 50 % in the calm corner.

Test 4:

The experiment was repeated in a somewhat modified form. The treatment remained the same. The damsons which were checked after 69 hours were placed on a wire mesh 15 cm high. In the damsons not exposed to the wind, 30 caterpillars (100 %) were dead, while in the aired damsons 10 out of 33 caterpillars (30 %) had succumbed. The difference in effect was particularly noticeable in this case.

The results of tests 3 and 4 prove that strong air movement greatly reduces the toxic effect of "E 605" against the caterpillars of the plum moth. Therefore a decrease in effect must be expected when faced with persistent strong winds during spray operations in the field. Light winds appear to have less influence on the result, otherwise differences would have been recorded in outdoor tests. It should be mentioned that the trees in the test field were growing in groups thus affording a better protection against the wind.

Test 5:

Once it had been established that the penetrative effect of the agent is obviously due to a vapour action, comparative sprays with two phosphoric acid esters each possessing a different vapour pressure were carried out late in the season on August 28, 1951.

Agent A contained 50 % of the active ingredient 0,0-diethyl thiophosphoric acid-0-p-nitrophenyl ester, and agent B 40 % 0,0-dimethyl thiophosphoric acid-0-p-nitrophenyl ester plus 10 % of the corresponding diethyl ester. Since the dimethyl ester has a higher vapour pressure, it is not at all improbable that its efficacy against the caterpillars of the plum moth is greater. A prerequisite would be to have the same toxic values, but these were not established.

At the late time the treatment was carried out (August 28), there were only a few caterpillars of the first stage still present. A number of the fully-grown caterpillars had already left the fruits. Most of the damsons were ripe for picking. One tree was sprayed with a 0.05 % wash of agent A and one with the same concentration of agent B. The high concentration was selected in order to be able to kill some of the caterpillars of the fifth stage, if necessary. An intermediate investigation after one day showed that the larval stages 2 to 4 were visibly injured by both agents while all the larvae of stage 5 moved normally. The difference determined after four days is shown in Table 20. Deserted fruits were not included in the evaluation. The number of young caterpillars found (stages 2 to 4) was too small in both cases to be able to recognize any differences in the success of the sprays. There is, however, a difference in the number of old caterpillars killed (L_5) whereby the dimethyl ester showed a partial effect which in the case of the diethyl compound had never been observed, even in preceding sprays.

It will be necessary to carry out further outdoor tests before being in a position to pass a final opinion on the increased effect of dimethyl ester.

Table 20. The effect of diethyl-p-nitrophenyl thiophosphate in comparison with the effect of dimethyl-p-nitrophenyl thiophosphate on the larvae of *Laspeyresia funebrana* Tr. in damsons 4 days following the treatment (active ingredient of the spray washes: 0.025 %).

	No. of larvae				Total number of larvae
	living		dead		
	L 2—4	L 5	L 2—4	L 5	
A. (diethyl ester)	1 (1)	15 (3)	5	1	26
B. (dimethyl ester)	(1)	16 (3)	8	8	36

In brackets: Additional number of visibly injured larvae.

4. Practical control measures

Preparations

In order to make sure when the second generation of the plum moth started flying, approximately 2000 fruits infested by the first generation were collected in the second half of June, and spread out on loose soil in four wooden boxes (40 × 40 × 12 cm) placed underneath a damson tree. On July 4, before the first

moths hatched, cardboard was nailed on the top of the boxes and they were lightly covered with garbage. Glass tubes were inserted on the sides to be able to catch the emerged imagoes every day. Hatching only lasted a few days (Fig. 5). Under the varying temperatures outdoors which are determined by soil coverage, nature of the soil and location, the moths could naturally have been on the wing for a long period. The first eggs were found, in conformity with the start of hatching, in the boxes on July 15.

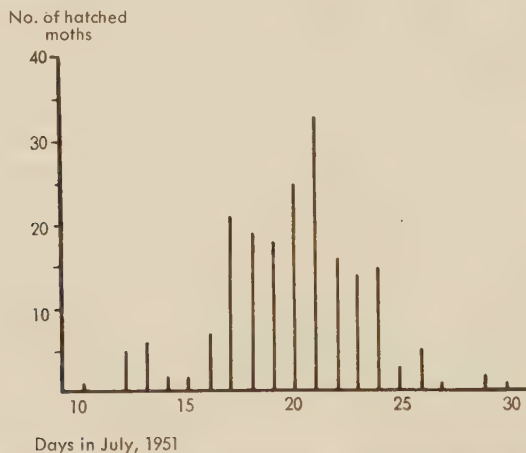


Fig. 5. No. of imagoes of *Laspeyresia funebrana* Tr. which emerged daily in the check boxes.

The results of the single sprays (Table 21) were obtained by evaluating each 4×50 damsons, whereby there were large differences from tree to tree in the same orchard.

When the dates the moths were on the wing and the experiences so far gained are taken into consideration, there is no difficulty in explaining the findings. The first spray on July 18 was carried out too early since only a small number of the eggs had been laid by then. After the spray, the infestation went up to 13 %. The sprays on July 27 and August 1 (Nos. 5 to 8) were carried out at a more favourable time. Apparently the flying period of the moths had come to an end. The caterpillars encountered were all still in the first stage, and they could, therefore, be eradicated to a satisfactory degree. Up till that time the fruits had barely been attacked by the caterpillars. The empty passages bored by the pests filled up with exudate, and it required a close examination to detect them at harvest time.

A single spray of 0.03 % "E 605" conc. applied at the right time is completely adequate to prevent damage being inflicted by the plum moth.

In order to fix the right dates for control during individual years, it is essential to keep a check on the time the pest is on the wing. The use of a few boxes from which the pests emerge to start flying only enables exact spray forecasts to be given for a small area since under complicated topographical conditions and with irregular soil coverage in a confined space large deviations have to be reckoned with in respect of the dates when the pest is flying. In

Table 21. Results obtained from the control of second generation *Laspeyresia funebrana* Tr. (1951) with 0.03 % "E 605".

No.	Dates of treatment	No. of evaluated trees	Number of fruits infested by eggs and larvae expressed in %			
			27. 7.	9. 8.	27. 8.	29. 8.
1	untreated	1	8 ± 3.1	24 ± 2.1	25 ± 1.9	
2	check	1	13 ± 2.8	19 ± 3.2	19 ± 3.4	
3	trees	1	7 ± 1.5	21 ± 2.8	23 ± 2.1	
4	18. 7.	3	10 ± 0.8	13 ± 1.0		
5	18. & 27. 7.	2	9 ± 2	2 ± 0.28		
6	27. 7.	1	12 ± 3	1 ± 0.4		1.2 ± 0.3
7	27. 7.	1	23 ± 1.5			1.3 ± 0.34
8	1. 8.	2	25 ± 2.1		26 ± 0.4	

addition to this, the flying dates do not give any indication as to the severity of the egg infestation. Even the use of baits has not satisfactorily solved the problem of establishing the dates the pest is on the wing (B ö h m 2).

It is advisable to wait with "E 605" sprays until most of the larvae have hatched out. It is a simple matter to establish when they do emerge since droplets of gum are often emitted from the passages as soon as the caterpillars have started their boring activities. The number of freshly laid eggs, also taking the temperature into consideration, gives an indication whether a further increase in egg laying can be expected. The control must be carried out before the caterpillars moult for the last time, firstly to prevent greater damage being inflicted, and secondly because the agent is not effective against the last larval stage. Many years of observations by T h i e m (35) and the investigations carried out by B o v e y (3) have shown that under similar climatic conditions the occurrence of the fifth larval stage can be expected in South West Germany during the first ten days of August. In observations made in Heidelberg in 1951, the first larvae of stage 5 were encountered on August 7. "Middle earlies" which ripen at the end of July to the beginning of August should be treated 10 days before the harvest. Two treatments at an interval of 10 to 14 days must be carried out when the pest occurs sporadically early in the season. This also applies to cases of severe infestation.

F. The effect of phosphoric acid esters on the larvae of the plum sawfly (*Hoplocampa minuta* Christ., *Hoplocampa flava* L.)

I. The importance of phosphoric acid esters in control work and their mode of action

Diethyl-p-nitrophenyl thiophosphate, like hexachlorocyclohexane and Quas-sia, is fully effective against the plum sawfly (T h i e m 29, 35). The most favourable time for application is after the crown leaves have fallen, although in field work this may be forgotten with the result that the larvae have already bored into the more or less well developed fruits before measures can be taken. In such cases it is most important to know up to which stage *Hoplocampa* larvae can be killed.

A large number of tests have shown that young larvae which are only a few days old can be completely eradicated with one spray of "E 605" conc. whereas many of the older stages are able to survive. From a few tests which were carried out, conclusions can be drawn in respect of the way in which diethyl-p-nitrophenyl thiophosphate acts against the pest. Here the two species *H. minuta* Christ. and *H. flava* L. occurred together. They were not investigated separately since their biology is alike.

At the time of the investigation (3 to 4 weeks after the blossom), the fruits were still small in comparison with those on which the behaviour of phosphoric acid esters, in respect to the control of *Laspeyresia funebrana* Tr., was observed. The penetrativity of the ester in respect to the effect on the larvae of the *Hoplocampa* species should, therefore, be much more perceptible.

Test 1:

Heavily infested mirabelle branches (Nancy mirabelle) were placed in water glasses on May 15 at a laboratory temperature of 20 to 22° C. 48 hours after they had been thoroughly treated with 0.03 % "E 605" conc., all the larvae, totalling 204, were dead (older stages).

Test 2:

This test was intended to check on the penetrativeness of the ester on infested plums treated on the side opposite the passage bored by the pest. The plums were picked, and their stalks were inserted through holes in a box into the water. Some of the plums were left untreated, some were treated on the half opposite the bored passage with 0.06 % "E 605" wash, and as a comparison others were coated all over with 0.03 % "E 605" wash. Here the low concentration was selected in order to apply the same quantity of active ingredient to a fruit as in the treatment with 0.06 %. Care was therefore taken in coating the fruits that none of the wash flowed into the bored passages.

After 90 hours there were no cases of 100 % mortality (Table 22) on the plums which were treated all over, as compared with the earlier laboratory tests. Probably this was due to the fact that in this test great care was taken that none of the agent flowed into the bored passages, which might quite

Table 22. The effect of "E 605" conc. on the larvae of *Hoplocampa minuta* Christ. and *H. flava* occurring in damsons, some of which were treated on the half opposite the bored passage, and the remainder treated all over.
Date of treatment: May 17, 1951.
Date of check: May 21, 1951.

	No. of infested fruits	Larvae		Fruits deserted by larvae
		dead	alive	
Untreated check	81	2	36	43
Treatment of fruits on one side with 0.06 % "E 605"	145	38	43	64
Treatment of whole fruits with 0.03 % "E 605"	133	63	10	60

possibly have happened in the preceding sprays. A smaller percentage of dead larvae was recorded on the fruits treated on only one side. The number of fruits deserted by the larvae was particularly high, this being due to natural conditions. Apparently the plums had absorbed more water than would have been possible under natural conditions when growing on the tree. The walls of the bored passages were extremely moist.

Test 3:

This test was carried out with the purpose of determining the penetrating power of the ester, using *Drosophila* flies. Plums were placed in water after being cut in half vertically in such a manner that the stalks remained whole and the plums could be kept fresh. A glass tube was affixed to each of 10 plum halves (five with a collodion solution and five with beeswax [Fig. 6]). 10 flies were inserted into each tube. The plum halves were coated three times with 0.05 % "E 605" conc. at intervals of 2 hours. The flies which fed on the plum juice showed no signs of injury whatsoever after 5 days. Therefore a penetration of the ester into the plums via the fruit meat could not be established in this case either, which would infer that "E 605" has a vapour effect on *Hoplocampa* larvae through the bored passages of the fruit.

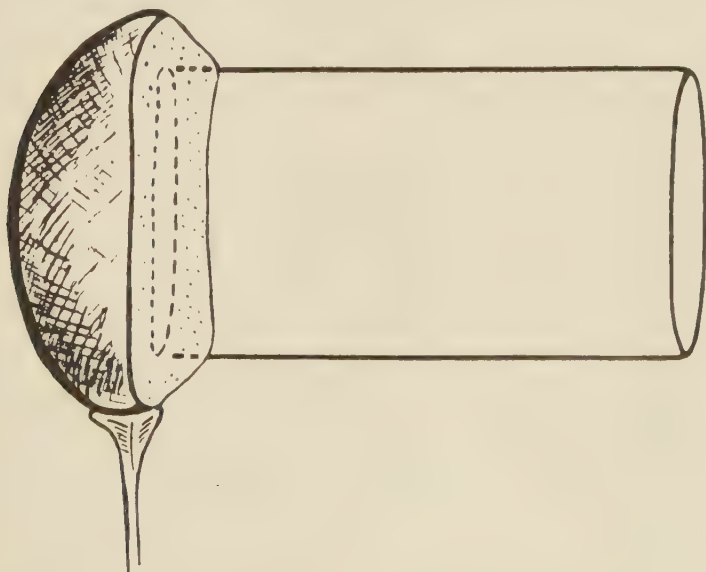


Fig. 6. Attachment of the glass tubes to the halved plums in the *Drosophila* test.

II. Practical control tests

Despite the thoroughness with which the sprays were applied, the outdoor tests (Table 23) did not come up to the standard of the laboratory tests in that the larvae in the fruits were not completely eradicated. This is due to the difference between the temperatures prevailing outdoors and those in the laboratory since it is an accepted fact that the agent is more effective at high temperatures (Dosse 4, Thiem 30, Lüdick 14). The mean temperatures outdoors were

3 to 4° lower. The possibility of a vapour action occurring is not only increased by the higher temperature but also by the considerably weaker air movement in the laboratory.

It is quite evident from the table that an increase of the concentration from 0.03 % to 0.05 % does not lead to a noticeable increase in the efficacy of the insecticide. A higher percentage of extermination was obtained in tests 5 to 8. Here the fruits of the investigated varieties, and in fact the larvae inhabiting them, were on the average smaller than those of the "Gute von Bry" and the "Wangenheims Early" varieties. Furthermore, the "Nancy" mirabelle and "Bühler" damson trees had a more compact crop. This would seem to indicate a more intensive vapour effect.

Concerning the practical application, it can be concluded that a thorough spray of 0.03 % "E 605" conc. is able at any time to stem damage inflicted by the *Hoplocampa* species. When assessing the infestation, it must be taken into consideration that each larva is capable of destroying anything up to five fruits.

Table 23. Effect of "E 605" conc. on the larvae of *Hoplocampa minuta* Christ. and *H. flava* L. occurring in several varieties of stone fruit. A 25 year old tree was treated in each case. The percentages of dead larvae together with the true mean errors are obtained from 4×50 evaluated larvae.

No.	Variety	Date of spray	"E 605" concentration	Quantity applied in litres	Date of check	Dead larvae expressed in %
1	"Nancy" Mirabelles	15. 5.	0.06	60	18. 5.	98 $\pm$ 0.82
2	"Gute v. Bry"		0.03	50	17. 5.	81 $\pm$ 2.9*
3	"		0.03	30	21. 5.	87 $\pm$ 4.2
4	"		0.05	30		83 $\pm$ 3.1
5	"Nancy" Mirabelles	18. 5.	0.03	30		96.5 $\pm$ 1.7
6	"		0.05	30		99 $\pm$ 0.58
7	"Bühler" damsons		0.03	30		89.5 $\pm$ 3.3
8	"		0.05	30		89.0 $\pm$ 3.7
9	"Wangenheims Early" damsons		0.03	30		63.5 $\pm$ 3.3
10	"		0.05	30		65.0 $\pm$ 3.4

* Checks: Dead larvae on May 18 : 87 $\pm$ 1.3 %
on May 19 : 83 $\pm$ 2.3 %

G. Summary

I. Object of the paper

The object of this paper was to closely investigate the effectiveness and mode of action of phosphoric acid esters, particularly of "E 605", on the different pests in respect to questions appertaining to practical control thus contributing to an explanation of the failures experienced in the past.

II. Preliminary investigations

"E 605" f and "E 605" conc., some of which had been in store for several years, did not show a loss of active ingredient in the biological test with *Calandra granaria* L. The *Drosophila melanogaster* M. used to trace "E 605" conc. are most resistant to the insecticide when they are at an age of 6 to 10 days.

III. The effect of phosphoric acid esters on *Rhagoletis cerasi* L.

1. Within 24 hours the active ingredient of "E 605" conc. penetrates through to the core of half-ripe and ripe cherries. Following an application of 0.05 % "E 605" conc. the maximum quantities of diethyl-p-nitrophenylthiophosphate present in the outer and inner layers of the fruit meat amounted to 1 mg/kg and 0.31 mg/kg respectively, but after a further three days the esters had dropped to below the detectable value. Greater quantities are retained in the peel. One day after the treatment with 0.03 % "E 605" conc. 50 to 75 mg/kg were found, after a further 1 to 2 days 3 to 4 mg/kg, and at the end of 17 days there were only slight traces present. The efficacy of the ester decreases somewhat quicker on green cherries than on ripe ones.
2. A thorough treatment with 0.03 % "E 605" conc. completely prevents a larval infestation developing from laid eggs. The insecticide does not, however, have a pronounced after-effect on eggs laid following the treatment.
3. When applied thoroughly, 0.03 % "E 605" conc. kills all larval stages. In a test carried out by Thiem — apparently due to the very large fruits — it was only possible to eradicate 80 % of the old larvae. The effect of "Systox" was similar.
4. Practical tests confirm that satisfactory results are obtained when "E 605" is applied 10 to 14 days before the harvest. The absolute ester residues in the fruits at harvest time following a treatment carried out 10 to 14 days beforehand are so slight that they represent no danger to the health of consumers.
5. In a large-scale test, the application of 0.03 % "E 605" conc. by atomizers at the rate of 2 to 4 litres per tree, preceded by a "DDT" treatment, reduced the expected larval infestation of 45 % to 2 %, and when "E 605" was used exclusively the infestation was reduced from 81 % to 3 %. 100 % eradication was attained with double the quantity of preparation.
6. The combined application of "DDT" and "E 605" offers promising possibilities of success. Treatment should be carried out about 3 weeks before the harvest.
7. "Systox" and "OMPA" prevent a larval infestation developing from laid eggs. An extension of the effect by 20 days as compared with "E 605" only occurs when higher concentrations are applied. Questions relating to toxicity to humans still have to be clarified.

IV. The effect of phosphoric acid esters on *Lespeyresia funebrana* Tr.

1. 0.03 % "E 605" conc. applied to damsons has an after-effect from 4 to 8 days on *Drosophila* flies. As the stage of ripeness progresses, the period of effectiveness lengthens. Contrary to the case of cherries, the ester cannot be traced in the fruit meat of damsons even when higher concentrations are applied. "E 605" spreads over the fruit peel of damsons only at an insignificant rate. 4 days following the application of 0.03 % "E 605" conc. the active ingredient residues on ripe fruits amounted to 0.74 mg/kg, and after 13 days to 0.38 mg/kg. Therefore there is no reason for concern about the health of consumers being impaired.

2. The contact effect on leaves (0.03 % "E 605" conc.) only lasts 2 to 3 days.
3. An average of 13 % viable larvae hatched out of plum moth eggs treated with 0.03 % "E 605" conc. There is no after-effect on eggs laid following a treatment.
4. 0.03 % "E 605" conc. eradicated 93 % of the first four larval stages. The fifth caterpillar stage was not injured (0.05 % "E 605" conc.). Several tests carried out revealed that "E 605" has a vapour effect on the larvae through the passages bored into the fruit.
5. One treatment with 0.03 % "E 605" conc. was sufficient to reduce the pest infestation to 1.2 to 2.6 % as compared with the untreated check of 19 to 25 %. The most favourable time for carrying out a control with "E 605" conc. is about the end of July in South West Germany, as soon as the first caterpillars of the second generation have penetrated into the fruit. It must be carried out at the latest before the caterpillars moult for the last time.

V. The effect of phosphoric acid esters on *Hoplocampa* species

1. Larvae of *H. minuta* and *H. flava* which had practically reached the fully-grown stage were killed to the extent of 64 to 97 % with 0.03 % "E 605" conc. Young larvae were eradicated completely.
2. A penetration of the ester into the fruit meat of damsons could not be established in this case either. The agent appears also to act on the larvae as a vapour through the bored passages.

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